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Abnormal light scattering detected by confocal biomicroscopy at the corneal epithelial basement membrane of subjects with Type II diabetes

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Abstract

Aims/hypothesis. Abnormalities of the basement membrane are thought to contribute to the complications of diabetes. The suitability of the cornea for detecting such abnormalities was assessed by determining its light-scattering index, a quantitative measure of tissue reflectivity in the basement membrane zone, with a confocal biomicroscope.
Methods. The light-scattering index was measured in 65 subjects with Type II (non-insulin-dependent) diabetes mellitus and 18 control subjects and was evaluated for its possible relation to the stage of diabetic retinopathy. Diabetic retinopathy was staged by ophthalmoscopic examination as non-diabetic (NDR), simple (SDR), preproliferative (PPDR), or proliferative (PDR).
Results. Examination of the cornea layer-by-layer with a confocal biomicroscope did not show any marked differences in morphology between diabetic and control subjects. The LSI (mean ± SD) was 0.81 ± 0.13, 0.87 ± 0.09, 0.90 ± 0.09, 0.90 ± 0.13, and 1.02 ± 0.25 in control subjects and in diabetic subjects with NDR, SDR, PPDR, or PDR, respectively; the light-scattering index of diabetic subjects with PDR was significantly greater than that of the control subjects ($p = 0.001$). An LSI greater than 1.0 was detected in 5.6, 6.3, 15.0, 15.4, and 50.0 % of control subjects and of patients with NDR, SDR, PPDR, or PDR, respectively; the percentage of subjects with an LSI greater than 1.0 was significantly increased in diabetic patients with PDR than for control subjects.
Conclusion/interpretation. These results suggest that the LSI increases with the stage of diabetic retinopathy, and that measurement of corneal light scattering could provide an index of basement membrane abnormality in people with diabetes. [Diabetologia (2001) 44: 340–345]

Keywords basement membrane, diabetes mellitus, cornea, confocal microscope, diabetic retinopathy.

Despite advances in the control of blood sugar concentration in subjects with diabetes mellitus the management of diabetic complications, such as retinopathy, neuropathy, and nephropathy remains a serious clinical problem. A common underlying feature in the pathology of these diabetic complications is an abnormality of the basement membrane, or basal lamina, in the affected tissues [1]. Thus, thickening of the basement membrane in various tissues, including renal glomeruli and tubules, vascular endothelium, perineurium, and bronchial and corneal epithelium has been described in subjects with diabetes mellitus [2–9].

The cornea is the outermost surface of the eyeball and has a relatively simple structure comprised of epithelial, stromal, and endothelial layers. The corneal epithelial basement membrane is located beneath the epithelium and does not contain any vascular elements. These anatomic characteristics of the cornea allow the epithelial basement membrane to be examined optically.

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Abbreviations: NDR, Nondiabetic retinopathy; SDR, simple diabetic retinopathy; PPDR, preproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; LSI, light-scattering index; TR, tissue reflectivity.

Table 1. Characteristics of the study subjects

Group	No. of subjects	No. of eyes	Age (years)	Sex (M/F)	Disease duration (years)	Fasting blood glucose (mmol/l)	HbA_{1c} (%)
Control	18	28	58.9 ± 19.2	12/6		5.0 ± 0.5	
NDR	16	30	60.9 ± 11.4	11/5	8.6 ± 5.3	9.6 ± 4.4	7.7 ± 2.2
SDR	20	38	60.3 ± 12.4	10/10	12.0 ± 7.4	8.7 ± 4.4	8.1 ± 1.9
PPDR	13	26	64.4 ± 6.7	8/5	18.0 ± 6.9	9.3 ± 4.6	9.2 ± 2.0
PDR	16	30	63.6 ± 10.1	10/6	15.9 ± 9.6	10.4 ± 4.5	7.5 ± 1.6
Diabetes total	65	124	62.1 ± 11.7	39/26	13.4 ± 8.1	9.5 ± 4.0	8.0 ± 2.0

Corneal complications in diabetes are well documented [10–12], although, unlike diabetic retinopathy, such diabetic keratopathy is not sight threatening. The corneal epithelium is well maintained in subjects with diabetes so long as the cornea is not injured. Once the cornea is damaged, however, the healing of epithelial wounds is often delayed and epithelial disorders persist. The underlying pathogenesis of diabetic keratopathy is thought to be attributable to an abnormality of the epithelial basement membrane [5, 6, 9, 12].

The slit-lamp microscope, which provides a sectional view of the cornea, has been used to examine corneal pathology in the clinical setting. The slit-lamp microscope, however, does not provide layer-by-layer images of the cornea. The recent development of the confocal biomicroscope [13–15] has allowed the cellular morphology of the cornea to be examined layer-by-layer [16–20]. One such instrument, the Confoscan (Tomey, Aichi, Japan), is equipped with the Z-Scan mode, which allows tissue reflectivity to be measured at each layer of the cornea, in addition to the morphological observation mode (Image mode). The results obtained with the Z-Scan mode can be presented either as the actual pattern of tissue reflectivity or in arbitrary units of light scattering. Such quantitative light-scattering data are quite likely related to the degree of opacity or structural disarray in each layer of the cornea. The advantages of the confocal biomicroscope include the facts that it is noninvasive and that it allows real-time observation in vivo, sequential observations in the same subject, and quantitation of tissue reflectivity.

To provide insight into the pathogenesis of diabetic complications due to basement membrane abnormality, we investigated whether it is possible, with the use of the confocal biomicroscope, to detect an abnormality in the basement membrane of the corneal epithelium in subjects with diabetes mellitus.

Subjects and methods

Subjects. This population based study comprised 65 subjects (39 men and 26 women) with diabetes (mean age ± SD, 62.1 ± 11.7 years; range, 25 to 83 years), in whom 124 eyes were examined, and 18 (12 men and 6 women) non-diabetic volunteers (mean age ± SD, 58.9 ± 19.2 years; range, 26 to 83 years), in whom 28 eyes were examined. No statistically significant difference in age or gender ratio was apparent between the diabetic and control groups. Only eyes without any past history of ocular trauma or ocular surgery were included in the study. Slit-lamp microscopy showed that all corneas studied were clear, and no epithelial abnormalities were observed by fluorescein staining. Informed consent was obtained from all subjects.

Endocrinologists in the Department of Third Internal Medicine, Yamaguchi University Hospital, diagnosed the diabetic patients with Type II (non-insulin-dependent) diabetes mellitus. On the basis of ophthalmoscopic examination and fluorescein angiography, retinal specialists in our department diagnosed the stage of diabetic retinopathy in these subjects according to the following classification [21]: nondiabetic retinopathy (NDR), simple diabetic retinopathy (SDR), preproliferative diabetic retinopathy (PPDR), or proliferative diabetic retinopathy (PDR). No statistically significant differences in the duration of diabetes, fasting blood sugar concentration, or HbA_{1c} value were detected among the various patient subgroups.

Confocal biomicroscopic observation. The corneas of all eyes of diabetic and control subjects that fulfilled the criteria for inclusion in the study were examined with a confocal biomicroscope. A drop of topical anesthetic (oxybuprocaine) was applied to each eye before examination. A pea-sized drop of a clear, viscous gel (Vidisic eye gel; Mann Pharma, Berlin, Germany) was placed onto the tip of the objective lens, which was then positioned on the center of the cornea. The morphology of the cornea was then examined layer-by-layer; the live image was monitored and recorded on videotape as the objective lens moved backwards and forwards along the *z*-axis.

Z-Scan and calculation of the light-scattering index (LSI). The Z-Scan mode of Confoscan provides an intensity profile of light scattering for all corneal layers, and a corresponding analysis allows measurement of scattering for individual layers. The objective lens is moved 1 mm forward and then back again within 1 s, and this movement is repeated continuously so that several profiles of light scattering can be recorded in a period of about 20 s.

The light reflected from each layer of the cornea is recorded continuously as tissue reflectivity (TR), a representative profile (Fig. 1). The data show the presence of three major peaks, which correspond to the interface between the tear film and the corneal epithelium, the interface between the epithelial basal cell layer and the anterior stroma, and the interface between the posterior stroma and the aqueous humor. The interface of the epithelial basal cell layer with the anterior stroma corresponds to the corneal epithelial basement membrane, or Bowman's layer.

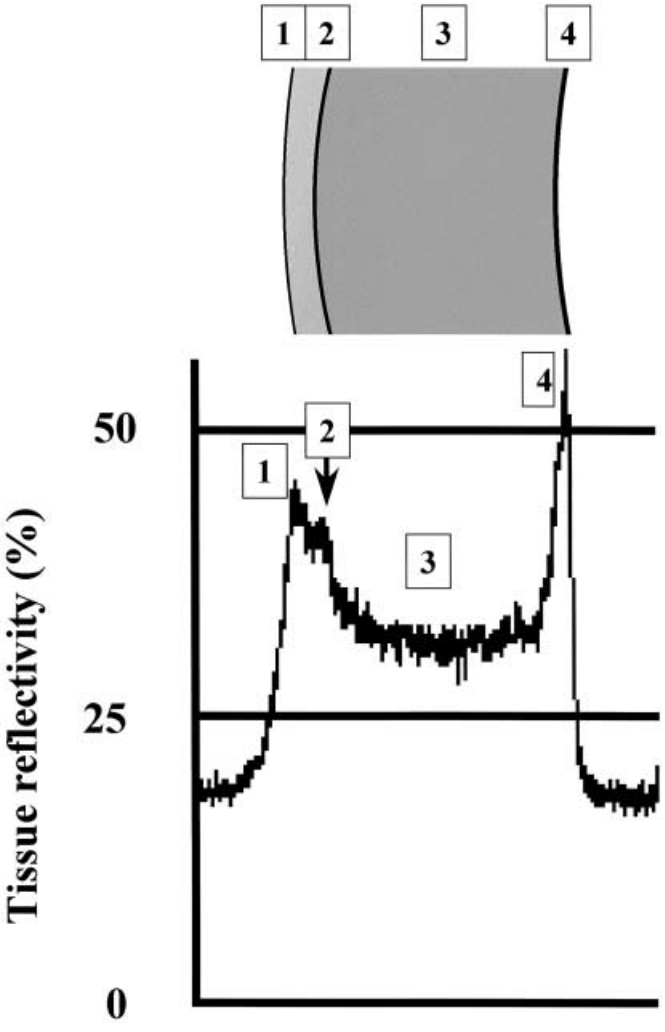

Fig. 1. Schematic diagram of the cornea (upper panel) and a representative pattern of corneal tissue reflectivity (TR) obtained with the Z-Scan mode of a ConfoScan confocal biomicroscope (lower panel). Numbers in both panels represent the following: 1, the interface between the tear film and the corneal epithelium (superficial epithelium); 2, the interface between the epithelial basal cell layer and the anterior stroma (epithelial basement membrane zone); 3, the stroma; and 4, the interface of the posterior stroma, Descemet's membrane, and the endothelium with the aqueous humor

To quantify the relative sizes of the first and second peaks of TR for each eye studied, we calculated the LSI, defined as the ratio of the intensity of the second peak to that of the first peak. The extent of TR generated from the gel was designated as the baseline value, because light scattering from the gel was relatively constant and low.

Statistical analysis. Data are presented as means ± SD and were analysed by Student's *t* test and the chi-square (χ^2) test. A *p* value of less than 0.05 was considered statistically significant.

Table 2. Comparison of the LSI between right and left eyes of study subjects

Group	No. of subjects	LSI		*p* value (*t* test)
		Right eye	Left eye	
Control	10	0.83 ± 0.15	0.83 ± 0.19	0.98
NDR	14	0.91 ± 0.12	0.90 ± 0.10	0.40
SDR	18	0.89 ± 0.10	0.84 ± 0.13	0.24
PPDR	13	0.91 ± 0.13	0.84 ± 0.16	0.21
PDR	14	1.01 ± 0.14	0.97 ± 0.14	0.23
Total	69	0.93 ± 0.13	0.89 ± 0.14	0.11

Results

Morphology. We examined the morphology of the corneal epithelium, epithelial basement membrane, stroma, and endothelium layer-by-layer in control subjects (Fig. 2, A through D) and subjects with diabetes (Fig. 2, E through H) with a confocal biomicroscope. At the layer of the epithelium, we observed the cobblestone-like appearance of the epithelial superficial cells (Fig. 2, A and E) and the honeycomb-like structure of the epithelial basal cell layer (Fig. 2, B and F), corresponding to the epithelial basement membrane, or Bowman's layer, of the cornea. Farther into the tissue, satellite (star-shaped) cells, or corneal fibroblasts, were observed in the stromal layer (Fig. 2, C and G); occasionally, nerve fibers were also detected in this layer. At the deepest plane, the honeycomb-like pattern of the endothelial cells was apparent (Fig. 2, D and H). The size and shape of the endothelial cells were relatively uniform, and the images of these cells were similar to those obtained with a specular microscope. No marked differences in the morphology of each layer of the cornea were apparent between non-diabetic and diabetic subjects.

With the Z-Scan mode of the confocal biomicroscope, we observed light scattering throughout the entire depth of the cornea. Representative Z-Scan profiles of TR for the corneas of control and diabetic subjects are shown in Figure 3. In most of the non-diabetic subjects, the first peak of TR (representing the interface between tear fluid and the surface of the epithelium) was higher than the second peak (representing the epithelial basement membrane zone). In contrast, in several diabetic subjects, the second peak of TR was higher than the first peak.

We hypothesized that the LSI should not differ between the two eyes of the same subject. We therefore compared the LSI values between right and left eyes in the 59 diabetic subjects and 10 non-diabetic control subjects in whom both eyes were examined. No statistically significant difference in the LSI was detected between the right and left eyes for all subjects, the control subjects, or the various subgroups of the diabetic subjects (Table 2). In the subsequent analysis, we therefore used the data from the right eye of subjects in whom both eyes were examined.

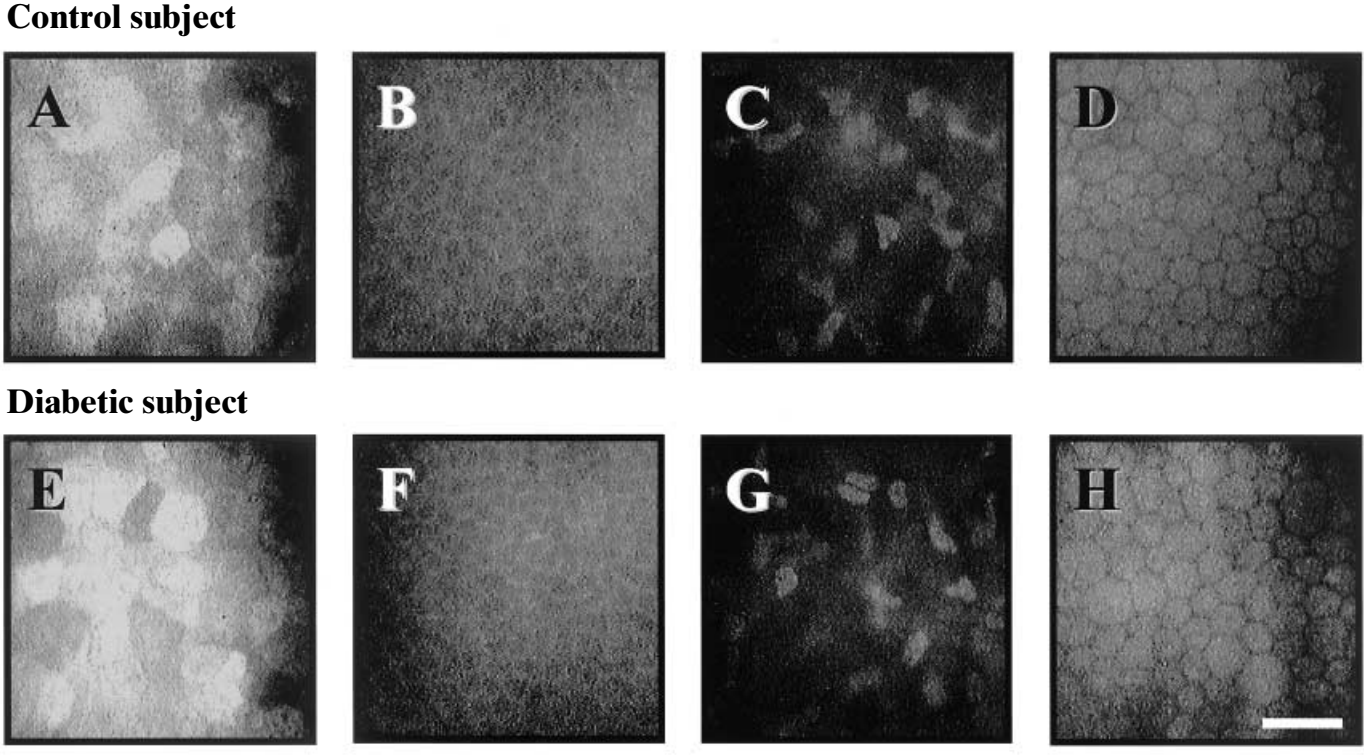

Fig. 2. Confocal biomicroscopic images of the corneas of a control subject (A through D) and a subject with Type II diabetes (E through H). (A and E) Superficial epithelial cell layer. (B and F) Epithelial basal cell layer, epithelial basement membrane, and anterior stroma. (C and G) Layer of the mid-stroma. (D and H) Layer of Descemet's membrane and endothelial cells. Scale bar, 50 μm

Table 3. LSI values for the cornea according to the stage of diabetic retinopathy (only the right eye was included for subjects in whom both eyes were examined)

Group	LSI	No. of eyes	*p* value vs control (*t* test)
Control	0.81 ± 0.13	18	
NDR	0.87 ± 0.09	16	NS[a]
SDR	0.90 ± 0.09	20	0.021
PPDR	0.90 ± 0.13	13	NS
PDR	1.02 ± 0.20	16	0.001

[a] NS, not significant

Next we analysed the relation between the LSI and the stage of diabetic retinopathy. The mean LSI increased with the severity of diabetic retinopathy, and follow a rank order of: control < NDR < SDR = PPDR < PDR (Table 3). Statistically significant differences in LSI were apparent between patients with PDR and either control groups ($p = 0.001$), patients with NDR ($p = 0.013$), or patients with SDR ($p = 0.022$), as well as between patients with SDR and control groups ($p = 0.021$). Finally, we compared the relative frequency of eyes with an LSI greater than 1.0 between the control subjects and the various patient subgroups; only the diabetic patients with PDR showed a statistically significant increase in this parameter (Table 4).

Discussion

The new technology of confocal microscopy and the transparent nature of the cornea have allowed us to observe changes in light scattering at the basement membrane zone of this tissue. The LSI of the cornea, a quantitative measure of light scattering at the basement membrane zone, was significantly increased in Type II diabetic subjects with PDR more than in non-diabetic control subjects. Furthermore, the LSI tended to increase with the severity of diabetic retinopathy in our study subjects. Of note all corneas examined were absolutely transparent and showed no evidence of pathology with a slit-lamp microscope.

A common underlying pathology of diabetic complications is an abnormality of basement membranes [6–8, 22, 23]. We hypothesized that an abnormal corneal epithelial basement membrane might scatter light even though the abnormality might not be detectable by routine clinical examinations. Our data are consistent with this notion. Whereas the LSI appeared related to the severity of diabetic retinopathy, it was not significantly correlated with the duration of diabetes, fasting blood glucose concentration, or HbA_{1c} value (data not shown). Our results therefore suggest that non-invasive examination of the cornea with a confocal biomicroscope might prove clinically useful for monitoring the severity of the epithelial

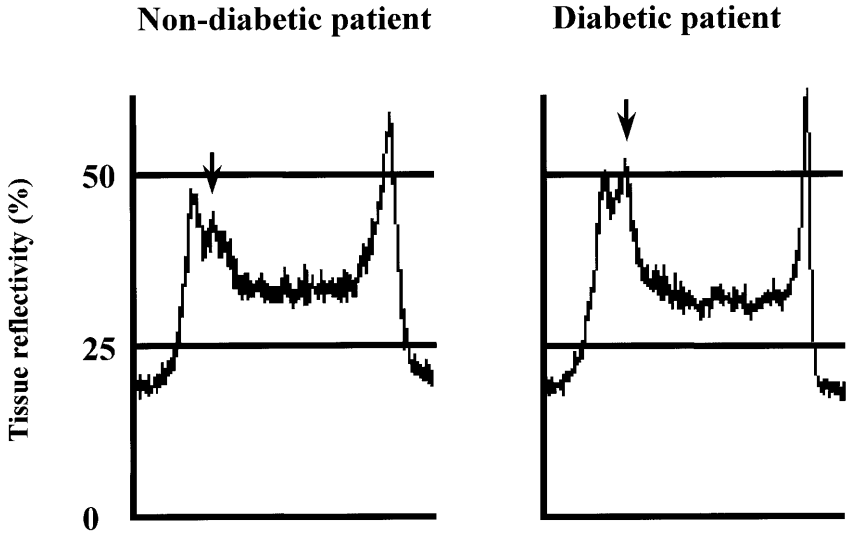

Fig. 3. Representative patterns of TR for the corneas of a control subject (left panel) and a Type II diabetic subject (right panel). In the control subject, the height of the first peak is greater than that of the second peak (arrow), whereas in the diabetic subject the converse is true

Table 4. Comparison of the number of subjects with corneas with an LSI of > 1.0 according to the stage of diabetic retinopathy (only the right eye was included for subjects in whom both eyes were examined)

Group	LSI > 1	LSI ≤ 1	Total	*p* value vs control (χ^2 analysis)
Control	1	17	18	
NDR	1	15	16	NS
SDR	3	17	20	NS
PPDR	2	11	13	NS
PDR	8	8	16	< 0.05

basement membrane abnormality in people with diabetes.

Given the low resolution of the current model of the confocal biomicroscope used in this study, we were not able to identify the corneal epithelial basement membrane definitively. Furthermore, the intensity of light scattering measured by the instrument is not an absolute value. The light-scattering profile of each subject yielded three peaks corresponding to the interfaces of the tear film with the epithelium, of the epithelium with the anterior stroma, and of Descemet's membrane and the endothelium with the aqueous humor. To normalize the changes in the relative intensity of light scattering in each individual cornea, we therefore calculated the ratio of the second peak to the first peak as the LSI, which should represent the extent of light scattering at the epithelial basement membrane relative to that at the surface of the corneal epithelium.

The Image mode of the confocal biomicroscope did not reveal any marked differences in morphology between the corneas of diabetic and control subjects. Whereas retinopathy is a vision-threatening complication of diabetes, corneal epithelial disorders do not arise in diabetic subjects so long as the cornea remains intact. If, however, the cornea is injured during surgery or other traumas, the healing of epithelial defects is slowed or, in some instances, fails to occur at all. The healing of corneal epithelial wounds is also delayed in rats with streptozotocin-induced diabetes [24]. The administration of fibronectin or hyaluronic acid promotes epithelial wound healing in these animals [24, 25], suggesting that a loss of adhesion molecules at the interface between corneal epithelial cells and the underlying substrate could contribute to the healing defect. The use of the confocal biomicroscope in clinical settings could allow the detection of subjects with early diabetic keratopathy.

The current model of the confocal biomicroscope used in this study scans a tissue thickness of 10 μm, whereas the thickness of the cornea is about 500 μm. At this low resolution, it is difficult to detect the morphological changes in the cornea of diabetic subjects that have been observed by electron microscopy [5, 6]. The current model of the confocal biomicroscope uses a halogen lamp. Thus, the development of an instrument that uses a laser should improve resolution and might increase the sensitivity of the LSI. Furthermore, determination of a standard value of LSI and of the relation between the LSI of the cornea and other measures of basement membrane abnormality in diabetic patients, such as peripheral nerve conductivity, will be required before LSI measurement becomes established for clinical evaluation of the basement membrane abnormality in subjects with this disease.

In conclusion, we have shown that the LSI of the cornea is related to the severity of diabetic retinopathy. If the basement membrane abnormalities in the various tissues and organs of the body affected by di-

abetes correlate with that in the cornea, then measurement of the LSI of the cornea might prove a simple, non-invasive means by which to assess the severity of diabetic complications other than retinopathy or keratopathy. Improvement in the sensitivity of the confocal biomicroscope should further increase its utility.

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