Serum Markers of Oxidative Stress and Severity of Diabetic Retinopathy

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OBJECTIVE — To compare serum markers of oxidative stress with diabetic retinopathy severity.

RESEARCH DESIGN AND METHODS — This cross-sectional study compared patients with types 1 and 2 diabetes with control subjects in western New York and Pennsylvania. Retinopathy severity was graded from funduscopic fields based on the Early Treatment of Diabetic Retinopathy Study. Serum samples were analyzed for thiobarbituric acid–reacting substances (TBARS), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities, creatinine, HbA_1, and triglycerides. Appropriate analysis of covariance models were performed.

RESULTS — TBARS ($P = 0.019$), triglyceride ($P = 0.004$), and glucose and HbA_1 (both $P < 0.001$) levels were elevated in diabetic patients compared with those in control subjects. SOD ($P = 0.003$) and GSH-Px ($P = 0.046$) levels were lower in diabetic patients than in control subjects. No correlation existed between SOD levels and either glucose or HbA_1 levels. No significant associations existed between levels of TBARS, SOD, or GSH-Px and severity of diabetic retinopathy. There was a significant association between poorer visual acuity and worse retinopathy ($P = 0.009$), which was only partly explained by macular edema.

CONCLUSIONS — Increased levels of TBARS and decreased levels of SOD and GSH-Px were found in diabetic patients compared with those in control subjects, but no significant associations were found between the levels of these substances and severity of retinopathy. When duration and type of diabetes and serum HbA_1 levels were taken into account, only visual acuity remained associated with more severe retinopathy.

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Oxidative tissue and organ damage may play roles in diabetes and its complications (1,2). Specifically, in diabetic retinopathy, both the genesis (3,4) and the advanced stage of proliferative diabetic retinopathy (PDR) have been hypothesized to be a result of increased oxidative species (5) or to be associated with ischemia-reperfusion injury at the boundaries of perfused and nonperfused retina (6), which leads to both increased oxidative species and neovascularization. In the rabbit, linoleic hydroperoxide, a reactive lipid end product of oxidative damage, when injected into the vitreous, led to neovascularization growing off of the retinal surface into the vitreous cavity (extraretinal neovascularization) (5). A study in humans suggested that antioxidant therapy with vitamin E might normalize diabetic retinal hemodynamics, known to be affected in preclinical retinopathy, and therefore might be important therapeutically in altering the course of diabetic retinopathy (7).

Vascular endothelial growth factor (VEGF), an angiogenic growth factor (8), has been found in the ocular fluid of patients with PDR (9,10). It is upregulated and released in response to ischemia (11) and oxidative products (12–14). In spontaneously diabetic rat retina, VEGF was significantly upregulated compared with that in control rat retina and correlated positively with serum-advanced glycation end products (12). The risk of diabetic retinopathy is also increased with poor glycemic control (15) and increased duration of diabetes. Elevated glucose causes a hypoxia-like imbalance by increasing the NADH-to-NAD ratio. This altered ratio has been hypothesized to be a mechanism for ischemic retinopathy (3) and a cause of increased production of the superoxide ion (16). Greater ischemia manifested by capillary nonperfusion of the retina leads to greater risk of extraretinal neovascularization and PDR, which is partly in response to upregulation and release of VEGF (11). Increased superoxide ion increases the oxidative load with greater reactive oxidative intermediates and advanced glycation end products, which also lead to increased release of VEGF (13,14) and the risk of neovascularization (5,8,9). Both ischemia and increased oxidation can lead to an increased production of lipid peroxides, which are themselves angiogenic (5).

Lipid peroxide levels measured in the thiobarbituric acid–reacting substances (TBARS) assay were increased in the serum of type 1 (21,22) and type 2 (17–20) diabetic patients. We wished to determine if there were associations between systemic oxidative compounds and antioxidant enzymes in patients with diabetes and with retinopathy severity. Advanced ischemia leads to PDR, which has blinding complications of vitreous hemorrhage and traction retinal detachment. We hypothesized that if increased levels of TBARS helped detect patients at a higher risk of developing PDR, then earlier or additional strategies might be instituted in managing PDR in these patients. Because metabolic control of the

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Abbreviations: ANCOVA, analysis of covariance; CV, coefficient of variation; ETDRS, Early Treatment Diabetic Retinopathy Study; GSH-Px, glutathione peroxidase; IRMA, intraretinal microvascular abnormalities; MAR, minimum angle of resolution; NPDR, nonproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; SOD, superoxide dismutase; TBARS, thiobarbituric acid–reacting substances; VEGF, vascular endothelial growth factor.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

patient (23) or diet (18) may affect levels of serum TBARS, we chose our sample from individuals within a delineated geographic area over a limited time as a means of reducing these possible variables.

RESEARCH DESIGN AND METHODS

Patients

The group with diabetes comprised patients who had either type 1 or type 2 diabetes for >5 years; duration of diabetes was defined as the time between diagnosis and the first visit. All subjects were recruited over an 18-month period from the medical clinic at Erie County Medical Center in western New York (clinic 1) and from a sole retinal practice providing care for retinal patients in northwestern Pennsylvania (clinic 2). Both clinics serve broad abutting geographic areas. We did not specifically assess the socioeconomic or educational status of the subjects. All subjects had ophthalmologic examinations, including stereoscopic fundus photography and fasting blood samples drawn within 2 weeks of the ophthalmologic examinations. All subjects participated in Institutional Review Board informed consent.

Diabetes was diagnosed as either a fasting serum glucose level >140 mg/dl (>7.77 mmol/l) on two separate occasions or an abnormal glucose tolerance test. Patients with secondary causes of diabetes or gestational diabetes were excluded. Hypertension was diagnosed as repeated blood pressure readings >140 mmHg systolic and/or >90 mmHg diastolic by report from the referring internist. Control subjects were healthy subjects who spanned a broad age range to encompass the likely ages of patients with both type 1 and type 2 diabetes. Control subjects were individuals recruited from clinic 1 who were being seen for routine medical examinations. Control subjects specifically did not have hypertension, diabetes, hyperlipidemia, cirrhosis, vascular disease, or evidence of eye disease, including retinovascular disease or best-corrected visual acuity <20/40. Fundus evaluations for control subjects specifically did not reveal hemorrhages, exudation, or neovascularization.

Ophthalmologic examinations

Ophthalmologic evaluations included visual acuity, intraocular pressure measurement (mmHg), anterior segment biomicroscopy, and dilated funduscopic evaluation.

Visual acuity

Best-corrected visual acuity was measured under similar lighting conditions by trained ophthalmic technicians and recorded as the minimum angle of resolution (MAR) expressed in decimal notation (e.g., 20/40 = 0.5, 20/100 = 0.2). The Early Treatment Diabetic Retinopathy Study (ETDRS) chart was used (24), and procedures to accurately measure visual acuity were practiced as outlined for current eye care guidelines for clinical research (25).

Funduscopic photography

Stereoscopic fundus photographs of the seven standard fields from the modified Airlie House classification (26) were taken of all subjects and judged for quality of content by an independent ophthalmic photographer. The stereoscopic fundus photographs were evaluated by a masked retinal specialist and graded for severity of diabetic retinopathy based on an abbreviated summary of ETDRS criteria (15,27, 28). Clinically significant macular edema was defined as the presence of retinal thickening and exudates determined by stereoscopic examination of the macular field as defined by ETDRS guidelines (29).

Study eye

The study eye was defined as that eye with the more severe retinopathy or the eye with the worse visual acuity if both eyes had equal degrees of retinopathy. If both eyes had equal levels of retinopathy and visual acuity, then the right eye was chosen.

Classification of retinopathy

Classification was based on fundus photographs primarily and on patient examination when stereoscopic information from photographs was limited because of the inability of a patient to adequately position in front of a fundus camera. The study eyes were graded on the basis of having nonproliferative diabetic retinopathy (NPDR) or PDR determined by the absence or presence of neovascularization growing off of the retinal surface and into the vitreous (PDR). The study eyes were graded as follows: 0, no retinopathy; 1, mild NPDR (mild hemorrhages, exudates, or cotton-wool spots); 2, moderate NPDR (moderate hemorrhages, mild intraretinal microvascular abnormalities [IRMA], or mild venous beading); 3, severe NPDR with either *1*) severe hemorrhages and/or microaneurysms in all four fields of fields 4–7 of the Modified Airlie House classification (26), *2*) venous beading in two fields of fields 4–7, or *3*) moderately severe IRMA in one field; 4, very severe NPDR (two or more of the above defining characteristics of severe NPDR); 5, early PDR (presence of extraretinal neovascularization less than high-risk character); 6, high-risk PDR (presence of extraretinal neovascularization of the disc equivalent to 0.25–0.33 disc area [28] or neovascularization elsewhere >1/2 disc area with vitreous or preretinal hemorrhage); and 7, PDR after panretinal photocoagulation (7).

Broader categories were created to reflect the risk of developing proliferative disease (28) and subsequent visual loss. These categories included patients with *1*) low risk (no retinopathy), *2*) moderate risk (mild and moderate NPDR), or *3*) advanced risk (severe NPDR, very severe NPDR, and all PDR).

Biochemical markers, TBARS, and antioxidant enzymes

All subjects had fasting blood samples obtained at the time or within 2 weeks of ophthalmologic evaluations. Samples were centrifuged at 3,000*g* for 10 min in the office, and the serum and packed cell pellets were frozen for transport to the laboratory for analysis.

Serum glucose (mmol/l), triglycerides (mmol/l), and creatinine (μmol/l) were measured with commercially prepared kits (Sigma, St. Louis, MO) on a Roche Cobas MIRA auto analyzer (Roche Diagnostics, Indianapolis, IN). Total glycosylated hemoglobin by percent hemoglobin (HbA_1) was calculated from 100 μl hemolyzed whole blood after chromatographic separation using the Sigma Diagnostic Kit 442. This procedure measures HbA_{1a}, $-A_{1b}$, and $-A_{1c}$ fractions and therefore is a higher value than the HbA_{1c} level alone. The following algorithm may be used to determine the value for HbA_{1c}: $HbA_{1c} = HbA_1 - (0.14/1.23)$ (15).

Levels of TBARS were measured from serum samples and expressed as micromoles per liter (30). Patient samples were run simultaneously with quality control samples of pooled serum from nonstudy normal healthy subjects. If analysis of the quality control TBARS level varied from ± 2 SD of a mean determined by a total of 60 runs, which included within-runs, between-day runs, and between-week runs, the analysis was deemed unacceptable and was repeated. The within-run coefficient of variation (CV) for the TBARS assay was 7.55%, and the day-to-day CV

was 9.2%. Quality control samples were retained for 90 days at −70ºC. New quality control samples were obtained with a 2-week overlap, during which old and new samples were run simultaneously and were found to fall within acceptable levels. The quality control samples were noted not to systematically increase over time. All samples were run within 3 h of blood draw.

Superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities were measured from the washed packed cell fraction based on a kinetic method (30) and units of activity normalized per milligram of hemoglobin.

Statistical evaluation

For analysis, grade of retinopathy was coded using the eight categories outlined above (0 [no retinopathy] to 7 [PDR after panretinal photocoagulation]) but was analyzed as a three-category variable (low-risk, moderate-risk, and advanced-risk). Analysis of covariance (ANCOVA) models were used to compare serum levels of biochemical markers between normal subjects and patients with diabetes after controlling for age, sex, and duration and type of diabetes. In addition, similar models were used for comparisons among type 1 diabetic patients, type 2 diabetic patients, and control subjects. The least significant difference method was used to compare individual groups after controlling for age and sex. The grade of severity was treated as a categorical variable (low-risk, moderate-risk, and advanced-risk), and regression analyses were run relating serum levels of biochemical markers against grade of retinopathy severity. Analyses of differences among retinopathy groups after controlling for age, sex, type and duration of diabetes, and serum HbA_1 levels were performed.

RESULTS

Demographics

There were 73 patients with diabetes and 26 control subjects (Table 1). The mean ± SD age of all patients with diabetes was 54 ± 15 years, and the mean age of control subjects was 45 ± 18 years. Of patients with diabetes, ~47% had duration of disease ≥15 years. Visual acuity was 20/40 or better in 62 (85%) of all patients with diabetes, 92% of type 1 diabetic patients, and 82% of type 2 diabetic patients.

Of type 1 and type 2 diabetic patients, 71 and 53%, respectively, had NPDR. Patients in the low or moderate categories comprised 50% of type 1 and 78% of type 2 diabetic patients, whereas those in the advanced category comprised 50% of type 1 and 22% of type 2 diabetic patients. PDR was present in 21% of type 1 and 12% of type 2 diabetic patients (Table 1). None of the patients with PDR had vitreous hemorrhage or traction retinal detachment. Gradable fundus photographs were present in 89 of 99 patients. Severity of retinopathy in the remaining 10 patients was determined according to an ophthalmologic evaluation by the retina specialist.

Table 1—*Demographics, medical history, and retinopathy of study subjects*

	Diabetic patients	Type 1 diabetic patients	Type 2 diabetic patients	Control subjects
n	73	24	49	26
Study population				
Clinic 1 (western NY)	33 (45)	10 (42)	23 (47)	26 (100)
Clinic 2 (western PA)	40 (55)	14 (58)	26 (53)	0
Age (years)				
11–40	16	13	3	10
41–70	51	11	40	14
71–100	6	0	6	2
Mean	54 ± 15	40 ± 10	60 ± 12	45 ± 18
Sex				
F	40 (55)	16 (67)	24 (49)	21 (81)
M	33 (45)	8 (33)	25 (51)	5 (19)
Duration of diabetes (years)				
<15	33 (45)	5 (21)	28 (57)	—
≥15	34 (47)	19 (79)	15 (31)	—
Unknown duration	6 (8)	—	6 (12)	—
Mean ± SD (*n*)	15.03 ± 9.62 (67)	21.06 ± 8.54 (24)	11.6 ± 8.5 (43)	—
Visual acuity				
<20/40	10 (14)	2 (8)	8 (16)	0
20/40–20/20	57 (79)	19 (79)	38 (78)	23 (88)
>20/20	5 (7)	3 (13)	2 (4)	3 (12)
Missing	1 (1)	0	1 (2)	0
Other conditions				
Hypertension	38 (52)	9 (37)	29 (59)	0
Clinically significant macular edema	7 (10)	1 (4)	6 (12)	0
Diabetic retinopathy (grade)				
No diabetic retinopathy	19 (26)	2 (8)	17 (35)	—
NPDR	43 (59)	17 (71)	26 (53)	—
Mild (1)	25 (34)	9 (38)	16 (33)	—
Moderate (2)	6 (8)	1 (4)	5 (10)	—
Severe (3)	11 (15)	6 (25)	5 (10)	—
Very severe (4)	1 (1)	1 (4)	0 (0)	—
PDR	11 (15)	5 (21)	6 (12)	—
Early (5)	3 (4)	0 (0)	3 (6)	—
High risk (6)	6 (8)	5 (21)	1 (2)	—
High risk treated with laser (7)	2 (3)	0 (0)	2 (4)	—
Retinopathy category (grade)				
Low risk (0)	19 (26)	2 (8)	17 (35)	—
Moderate risk (1 and 2)	31 (42)	10 (42)	21 (43)	—
Advanced risk (3–7)	23 (32)	12 (50)	11 (22)	—

Data are *n*, means ± SD, or *n* (%) unless otherwise indicated.

Antioxidant enzymes, TBARS, serum values, and diabetes

To determine whether levels of serum TBARS, antioxidant enzymes, or other biochemical markers differed between patients

Table 2—*Antioxidant enzymes, TBARS, and serum values in subjects*

	Diabetic patients	Control subjects	*P**
Serum value			
TBARS (mmol/l)	2.61 ± 1.22 (72)	1.93 ± 0.57 (26)	0.019†
SOD (U/mg Hb)	10.0 ± 3.4 (71)	12.5 ± 3.5 (26)	0.003†
GSH-Px (U/g Hb)	47.5 ± 15.2 (71)	55.6 ± 20.6 (26)	0.046†
HbA_1 (% Hb)	10.1 ± 2.1 (69)	8.1 ± 1.1 (26)	<0.001†
Glucose (mmol/l)	11.1 ± 5.7 (72)	4.7 ± 0.6 (26)	<0.001†
Creatinine (μmol/l)	79.8 ± 28.0 (72)	72.5 ± 17.0 (26)	0.37
Triglycerides	1.81 ± 1.73 (72)	0.90 ± 0.47 (26)	0.004†
Age	54 ± 15 (73)	45.2 ± 18 (26)	0.020†
Visual acuity ‡	0.82 ± 0.3 (72)	0.96 ± 0.17 (26)	0.08

Data are means ± SD (*n*). **P* value is for comparison between diabetic patients versus control subjects in analysis of covariance model after controlling for age and sex. †Significance ($P < 0.05$). ‡Measured in the eye with the more severe retinopathy or the eye with the worse visual acuity; if both eyes were equal, then the right eye was measured.

with diabetes and control subjects, an ANCOVA between all patients with diabetes and control subjects was created after controlling for age and sex. Levels of TBARS ($P = 0.019$) were significantly higher in patients with diabetes versus those in control subjects ($P = 0.019$), as were levels of HbA_1 ($P < 0.001$) and triglycerides ($P = 0.004$) (Table 2). Antioxidant enzyme activities of SOD ($P = 0.003$) and GSH-Px ($P = 0.046$) were significantly lower in patients with diabetes versus those in control subjects.

When patients with diabetes were classified as type 1 and type 2 diabetic patients and compared with control subjects, the overall ANCOVA, after controlling for age and sex, was significant for SOD ($P = 0.004$) (data not shown). Subgroup analysis indicated that mean SOD levels in both type 1 and type 2 diabetic patients were significantly lower compared with those in control subjects ($P = 0.005$). In addition, HbA_1 and glucose levels were significantly elevated in type 1 and type 2 diabetic patients compared with those in control subjects ($P < 0.001$). The overall analyses of covariance for levels of TBARS ($P = 0.099$), GSH-Px ($P = 0.11$), and creatinine ($P = 0.66$) were not significant.

Because Cu-Zn-SOD function may be affected by nonenzymatic glycation (31,32), we wished to determine if the serum level of SOD function was correlated with either glucose or HbA_1 levels. When all patients with diabetes were analyzed, glucose and HbA_1 levels were significantly correlated ($r = 0.32$, $P = 0.007$, $n = 69$); however, neither SOD and HbA_1 levels ($r = -0.24$; NS, $n = 69$) nor SOD and glucose levels were significantly correlated ($r = -0.14$, NS, $n = 71$). When patients with type 1 diabetes were evaluated, only SOD and HbA_1 levels were significantly correlated ($r = -0.59$, $P = 0.002$, $n = 24$); in this subgroup, glucose and SOD levels were not correlated, and, therefore, the analysis was less meaningful. In patients with type 2 diabetes, only glucose and HbA_1 levels were significantly correlated ($r = 0.53$, $P < 0.001$, $n = 45$).

Antioxidant enzymes, TBARS, serum values, and diabetic retinopathy

To determine whether serum TBARS and antioxidant levels were associated with severity of retinopathy, an ANCOVA that compared category or grade of retinopathy

Table 3—*Antioxidant enzymes, TBARS, and serum values as associated with retinopathy severity in subjects with diabetes*

	Low risk	Moderate risk	Advanced risk	Moderate versus low risk (*P*)	Advanced versus low risk (*P*)	Overall ANCOVA (*P*)
Serum value						
TBARS (mmol/l)	2.76 ± .89 (18)	2.33 ± 1.19 (31)	2.86 ± 1.45 (23)	A 0.50	A 0.50	A 0.22
				B 0.33	B 0.44	B 0.086
SOD (U/mg Hb)	10.0 ± 4.5 (18)	9.7 ± 3.1 (30)	10.4 ± 2.8 (23)	A 0.59	A 0.86	A 0.64
				B 0.49	B 0.93	B 0.67
GSH-Px (U/g Hb)	47.2 ± 17.4 (18)	47.8 ± 14.8 (30)	47.4 ± 14.6 (23)	A 0.42	A 0.52	A 0.72
				B 0.41	B 0.40	B 0.66
HbA_1 (% Hb)	9.9 ± 2.6 (18)	10.3 ± 2.2 (29)	10.0 ± 1.6 (22)	A 0.61	A 0.38	A 0.67
Glucose (mmol/l)	10.3 ± 3.4 (18)	10.2 ± 3.8 (31)	12.8 ± 8.4 (23)	A 0.27	A 0.93	A 0.35
				B 0.36	B 0.73	B 0.26
Creatinine (μmol/l)	67.4 ± 23.0 (18)	80.2 ± 21.0 (31)	89.2 ± 36.2 (23)	A 0.50	A 0.10	A 0.20
				B 0.47	B 0.17	B 0.37
Log triglycerides	0.56	0.13	0.41	A 0.28	A 0.74	A 0.17
				B 0.26	B 0.71	B 0.15
Triglycerides	2.36 ± 2.35 (18)	1.32 ± 0.81 (31)	2.04 ± 1.98 (23)	A 0.43	A 0.39	A 0.10
				B 0.41	B 0.36	B 0.09
Age	57.8 ± 10.0 (19)	53.3 ± 13.3 (31)	50.5 ± 19.3 (23)	—	—	—
Visual acuity	0.97 ± 0.20 (19)	0.82 ± 0.27 (31)	0.68 ± 0.36 (22)	A 0.081	A 0.002	A 0.006
				B 0.16	B 0.003	B 0.009

Data are means ± SD (*n*), unless otherwise indicated. A, Determined after controlling for age, sex, and duration and type of diabetes; B, determined after controlling for age, sex, duration and type of diabetes, and HbA_1.

status (low, moderate, or advanced risk) in control subjects and diabetic patients was run after controlling for age, sex, and duration and type of diabetes. The data on categories are presented, because it is believed the data accurately reflect ischemia and, thus, risk of PDR and visual loss (Table 3). A trend toward highest TBARS values in the advanced group being associated with more severe retinopathy was found after controlling for age, sex, duration and type of diabetes, and HbA_1 levels ($P = 0.086$).

When all patients with diabetes were segregated according to type and analyzed by category, HbA_1 levels were significantly higher in type 1 diabetic patients with more severe retinopathy ($P = 0.004$; overall ANCOVA) (data not shown).

Visual acuity

There was a trend toward worse visual acuity in patients with diabetes compared with control subjects after controlling for age and sex ($P = 0.08$) (Table 2). When category of severity of retinopathy was related to visual acuity, the overall ANCOVA was significant after controlling for age, sex, type and duration of diabetes, and HbA_1 levels ($P = 0.009$) (Table 3). Subgroup analysis showed a significant difference between the categories of advanced versus low retinopathy risk ($P = 0.003$). Results were similar after removing patients with macular edema, although the sample size was reduced by seven subjects.

CONCLUSIONS —

We found that levels of TBARS were significantly elevated in patients with diabetes compared with those in control subjects, which supports the findings by others (18–23,33). In our patients, the TBARS levels were lower than those reported in other studies (18) and may reflect genetic or dietary differences (17–22). Our samples come from two similar abutting geographic regions (western New York and Pennsylvania) and were collected over a limited recruitment time. We attempted, without imposing too many exclusion criteria, to reduce the possible variability from dietary or metabolic (18,23) factors that may be present when sampling patients from broad geographic locations over extended periods of time.

We were unable to support the hypothesis of an association between severity of diabetic retinopathy and TBARS as a measure of oxidative stress, although a trend was noted after controlling for age, sex, type and duration of diabetes, and HbA_1 levels. Detection of lipid peroxidation by methods other than by assaying TBARS may elucidate a serum marker associated with retinopathy severity. TBARS levels measured in the red blood cell membrane were found to vary depending on the fatty acid and cholesterol concentration in the membrane and therefore did not consistently and accurately provide information about antioxidant defense systems (34). In addition, lipid peroxides represent only one component of TBARS. Elevation of any one lipid peroxide may be angiogenic (5), but it may be masked in the overall TBARS level. In future studies, specific characterization of the peroxy and hydroxy derivatives of lipid peroxides by high-pressure liquid chromatography (35) may be indicated to more carefully assess possible associations with retinopathy severity. In addition, this study had a relatively small sample size and may have insufficient power to detect an association.

We found a strong association between reduced SOD activity and diabetes, both in type 1 and type 2 diabetic patients. This would seem unexpected because in a disease with elevated oxidative compounds, a compensatory increase in antioxidant enzymes would be desirable. Vascular endothelial cells exposed to oxidative stress by low nonlethal concentrations of hydrogen peroxide changed their expression of antioxidant levels, which suggests an adaptive rather than a deleterious phenomenon (36). SOD provides the major mechanism to scavenge the superoxide radical. The literature provides conflicting evidence of serum SOD and diabetes. Some studies have reported reduced levels of SOD activity (37,38), whereas others found no association between diabetes and either SOD or GSH-Px levels (19,20,39). Because nonenzymatic glycation of one form of SOD, Cu-Zn-SOD, can cause it to undergo fragmentation (31) and can lower its activity (32), we tested whether there was a correlation in serum glucose levels or HbA_1 and SOD activity. Such a correlation might provide support that SOD activity was reduced by elevated glucose. Although glucose and HbA_1 levels were correlated, neither SOD and glucose levels nor SOD and HbA_1 levels were correlated. In the subgroups of type 1 and type 2 diabetic patients, the numbers were small and the results less conclusive. These findings suggest that an association among high serum glucose, HbA_1, and reduced SOD activity does not exist. Perhaps SOD activity is altered in diabetes in another way, such as by reduced production of SOD. To test this theory, SOD and protein activity can be assayed. We found no association between severity of retinopathy and GSH-Px activity (Table 3).

We believe that analysis by category of retinopathy severity (no retinopathy as low risk, NPDR less than severe as moderate risk, and severe NPDR or worse [including PDR] as advanced risk) would more accurately reflect levels of ischemic damage to the retina. However, this categorization may miss other important factors that individual grouping reflects. An association with nephropathy and high TBARS levels was found in diabetic patients (40). SOD and catalase levels were lowest in these patients with nephropathy. High serum triglyceride and HbA_1 levels were reported as risk factors for high-risk proliferative retinopathy in a study of patients from the ETDRS (41). We did not find HbA_1 levels to be significantly associated with worse retinopathy, except when patients with diabetes were segregated according to type and then analyzed. There was an association with higher HbA_1 levels and more severe retinopathy in type 1 diabetic patients only.

We found that visual acuity, when used as a continuous variable, was strongly associated with more severe retinopathy after controlling for age, sex, and type and duration of diabetes. The significance was found when analysis was run according to group or category of retinopathy severity. An association between more severe retinopathy and reduced visual acuity was also found in patients studied in the ETDRS (41). Clinically significant macular edema, though present in 10% of patients with diabetes and accountable for some of the visual impairment in epidemiological studies of diabetic retinopathy (42), was not the sole factor associated with this vision loss. When patients with clinically significant macular edema were removed from the analysis, visual acuity was still reduced, which indicates vision loss for other reasons. Age-related and diabetes-associated cataract (43) and, possibly, changes in the photoreceptors (44) or microvasculature may be contributory (45,46). Even when HbA_1 was controlled for, visual acuity was the only variable that remained associated with worse retinopathy.

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