Prevalence of Diabetes Mellitus and Correlation of Urinary Transforming Growth Factor-β_1 with Blood Hemoglobin A1C in the Atascosa Diabetes Study

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Introduction: This study was conducted to determine the prevalence of type 2 diabetes and prediabetes in the Atascosa Diabetes Study sample and to ascertain the relationship between urinary transforming growth factor-β_1 (TGF-β_1) and blood hemoglobin (Hgb) A1C.

Methods: Subjects (N=526) classified as adjusted normal, at risk, prediabetes, and diabetes mellitus were given a one-hour and two-hour postprandial glucose (PPG) test. Morning urine samples were collected to test for a correlation of TGF-β_1 with blood HgbA1C.

Results: Of the subjects, 14.3% had diabetes, 31.6% had prediabetes, 7.9% were at risk, and 46.2% were adjusted normal. Sensitivity and specificity for one-hour PPG for prediabetes and diabetes were significant, with an efficiency of 80.2%–90.9% and a likelihood ratio of 4.7–10.2. Receiver operating characteristic analysis resulted in an area under the curve of .880±.016 for one hour to prediabetes and diabetes and .960±.016 for one hour to diabetes. Prediabetes was 1.07 times more prevalent in Hispanics, but diabetes was 1.65 times greater in Whites. Urinary TGF-β_1 was more than fivefold higher in poorly controlled versus controlled diabetic or normal subjects and had a significant positive correlation with HgbA1C.

Conclusions: The percentage of subjects with type 2 diabetes was 1.64 times higher than the national average. Prevalence of prediabetes was equivalent in Hispanics and Whites, and the reversal for diabetes might reflect higher mortality rate from diabetes in Hispanics in Atascosa County. Use of one-hour PPG and urine markers for early kidney involvement could improve this disparity in such high-risk populations. (*Ethn Dis*. 2008;18[Suppl 2]:S2-54–S2-59)

Key Words: Cytokines, Diabetic Nephropathy, Hispanic, Glucose Tolerance

Introduction

The Atascosa Health Center (AHC) is a rural clinic in Pleasanton, Texas. The Atascosa Diabetes Study (ADS) was established to evaluate the diabetes epidemic among the population served by the AHC and to identify a possible biomarker for diabetic nephropathy among the served population, which is ≈75% Mexican American and 24% White.

The Mexican American population is genetically predisposed to higher risk for type 2 diabetes mellitus.[1,2] The growing rate of obesity in the United States has coincided with an increasing prevalence of type 2 diabetes,[3] suggesting that the Mexican American population living on the southern US border is at greater risk.

Diabetes is a primary cause of nephropathy, which is a leading cause of death in diabetes patients.[4,5] Increased glomerulosclerosis and proteinuria are associated with plasma and urine levels of transforming growth factor-β_1 (TGF-β_1), a cytokine activated by high glucose levels that causes initial structural damage to glomeruli.[6,7] Blood hemoglobin A1C (HgbA1C), a test for control of glucose levels in diabetic patients over time, is useful for monitoring diabetes control. Chronically high levels of glucose cause progressive damage to the kidney, which is monitored by presence of albumin in urine. However, by the time persistent albuminuria exists, kidney damage has occurred. Increased levels of TGF-β_1 in urine can more accurately predict early diabetic nephropathy.[6,7] Therefore, this study assessed prevalence of type 2 diabetes at various stages of development and correlated urine TGF-β_1 with blood HgbA1C levels for patients with controlled and poorly controlled diabetes.

Methods

ADS enrolled a self-selected group of patients at the AHC for whom the oral glucose tolerance test (OGTT) included fasting blood glucose (FBG) and a one-hour and two-hour postprandial blood glucose (PPG) determination. Patients of the AHC who were ≥18 years old, not currently pregnant, and not previously diagnosed with diabetes or prediabetes were asked to allow access to their records to be used as the sample for the present study. Subjects also approved use of the routine urine sample for additional testing of TGF-β_1. Body mass index (BMI) could not be calculated for two subjects. The AHC review board approved this study. Data were handled according to Health Insurance Portability and Accountability Act regulations to protect patient confidentiality.

AHC medical records verified that the sample accurately represented the overall patient population. A pseudo-random number generator was used to generate random chart numbers. Patient demographics were recorded, including sex, date of birth, ethnicity, height,

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weight, and age, and BMIs were calculated. If a chart number was missing or the patient was <18 years old or pregnant, the next highest chart number was used. BMI could not be calculated for six subjects. For urine-blood correlation, the original sample population was 98; 21 were White and 77 Hispanic. The HgbA1C levels were available for only 48 of these subjects. This study group comprised 27 normoglycemic nondiabetic participants, 13 participants with controlled diabetes, and 8 participants with poorly controlled diabetes. Their ages ranged from 4 to 82 years.

The ADS sample was categorized by four diabetic states. Ranges were based on American Diabetes Association diagnostic criteria'[8] with an additional ''at risk'' category for this study. The classifications were adjusted normal (FBG <100 mg/dL, one-hour PPG <170 mg/dL, two-hour PPG <130 mg/dL), at risk (one-hour PPG ≥170 mg/dL, two-hour PPG 130–139 mg/dL), prediabetes (FBG 100–125 mg/dL, two-hour PPG 140–199 mg/dL), and diabetes mellitus (FBG ≥126 mg/dL, two-hour PPG ≥200 mg/dL). Each subject produced three glucose concentration data points; the highest categorical value was used for classification.

To determine a one-hour concentration range to be considered at risk, a sensitivity-specificity analysis was performed between one-hour PPG and diabetic state, either FBG or two-hour PPG. First, one-hour PPG was treated as the screening test, and prediabetes or diabetes was treated as the disease. Thus, disease was considered to be the lower limit of the prediabetes range (either FBG ≥100 mg/dL or two-hour PPG ≥140 mg/dL). A subject with both values lower (ie, FBG <100 mg/dL and two-hour PPG <140 mg/dL) was considered negative for disease. Second, analysis was performed to relate one-hour PPG to diabetes only (without inclusion of the prediabetes range). Thus, disease was considered to be the lower limit of the diabetic range (either FBG ≥126 mg/dL or two-hour PPG ≥200 mg/dL). A subject with both values lower (ie, FBG <126 mg/dL and two-hour PPG <200 mg/dL) was considered negative for disease. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and likelihood ratio were calculated.[9] Efficiency was defined by determining the value along the x-axis, which corresponded to greatest accuracy (fewest false-negative and false-positive results). Receiver operating characteristics (ROC) and area under the curve (AUC) were calculated by using MedCalc for Windows, version 9.3.7.0 (MedCalc Software, Mariakerke, Belgium).

OGTT consisted of FBG measurement, followed within five minutes by oral ingestion of 100 g glucose in a 10-oz OGTT beverage (Fisher HealthCare, Houston, Texas). Blood for FBG measurement was drawn via finger-prick, while samples for one-hour and two-hour measurements were via venipuncture. Glucose concentration was measured in milligrams per deciliter with the Accu-check Advantage system (Roche Diagnostics, Indianapolis, Ind).

Subjects collected first morning urine sample in a sterile container. Samples not tested on the day of collection were stored at 4°C. Subjects were excluded if the time since prior urination was less than four hours, the subject was pregnant, had kidney disease, or produced samples that tested positive for blood.

Measurement of urinary TGF-β_1 was performed with a Quantikine immobilized receptor assay (R&D Systems, Minneapolis, Minn). The supernatants of urine specimens centrifuged at 200xg for 10 minutes were acid activated and neutralized according to the manufacturer's protocol. A 200-µL urine aliquot was incubated on the TGF-β_1 receptor-coated plates for three hours. Bound TGF-β_1 was detected by using a polyclonal anti-TGF-β_1 horseradish peroxidase conjugate and the substrate tetramethylbenzidine. Absorbance was read at 450 nm. Measurements of plasma HgbA1C were conducted at fasting by a turbidimetric immunoinhibition method using a Synchro CX5 Analyzer (Beckman Instruments, Fullerton, CA).

Statistical analyses were conducted to detect differences between the ADS sample and AHC sample, and between the four diabetic categories. We used χ^2 tests and Fisher exact tests to test for significance level of differences in sex and ethnicity. One-way analysis of variance (ANOVA) and post hoc tests (Dunnett) were conducted for significance levels of differences in age and BMI. Urine data were analyzed by ANOVA followed by Newman-Keuls Multiple Comparison Test. A linear correlation test was used for TGF-β_1 and HgbA1C. Significance level was set at $P<.05$.

RESULTS

Demographics of the ADS sample were compared to the sample group taken from AHC records. Frequency of demographic factors of sex and ethnicity were similar in both groups (Table 1). Slight differences were detected between the ADS sample and the AHC group in distribution of age and BMI.

The mean age of subjects in the adjusted normal category was significantly lower than for the prediabetes and diabetes categories ($P<.01$) (Table 2). The mean ages of the at risk, prediabetes, and diabetes categories were not significantly different from each other ($P>.05$). Additionally, the mean BMI for the adjusted normal category was significantly lower than that of the prediabetes and diabetes categories ($P<.01$). The mean BMI in the at risk category was not significantly different from any of the other three categories. The mean BMI in the diabetes category was significantly high-

Table 1. Demographic distribution of the subjects in the Atascosa Diabetes Study (ADS) and a sample from the Atascosa Health Center (AHC)

Characteristic	ADS Sample (*N*=526) *n* (%)	AHC Sample (*N*=340) *n* (%)
Sex		
Female	384 (73.0)	255 (75.0)
Male	142 (27.0)	85 (25.0)
Ethnicity		
Hispanic	406 (77.2)	268 (78.8)
White	116 (22.1)	71 (20.9)
Black	4 (.8)	1 (.3)
Age, years		
Mean (±SD)	41.61 (±13.1)	38.69 (±15.4)
<20	16 (3.0)	17 (5.0)
20–29	92 (17.5)	105 (30.8)
30–39	137 (26.1)	72 (21.2)
40–49	132 (25.1)	60 (17.7)
50–59	90 (17.1)	45 (13.2)
≥60	59 (11.2)	41 (12.1)
BMI, kg/m^2		
Mean (±SD)	34.52 (±7.5)	31.34 (±8.03)
<18.5	4 (.8)	5 (1.4)
18.5–24.9	35 (6.7)	71 (20.9)
25–29.9	117 (22.1)	90 (26.5)
30–34.9	151 (28.7)	75 (22.1)
35–39.9	105 (19.9)	53 (15.6)
≥40	112 (21.3)	40 (11.8)
Incalculable	2 (.4)	6 (1.7)

SD = standard deviation, BMI = body mass index.

er that than of the prediabetes category ($P<.01$).

For one-hour PPG to prediabetes/diabetes analysis, the most efficient concentration is 158 mg/dL. Sensitivity and specificity at this point are 77.2% and 83.2%. At this cutoff point, the likelihood ratio is 4.58. At 160 mg/dL, sensitivity and specificity are 75.1% and 83.9%, with a likelihood ratio of 4.65. For one-hour PPG to diabetes analysis, the most efficient concentration is 209 mg/dL. Sensitivity and specificity at this point are 88.0% and 91.4%. At this cutoff point, the likelihood ratio is 10.18. At 210 mg/dL, sensitivity and specificity are 85.3% and 91.6%, with a likelihood ratio of 10.13.

Because calculation of PPV and NPV is dependent on the prevalence of disease in the sample,[8] Table 3 shows PPV and NPV of one-hour PPG to diabetes test for three prevalences: American Diabetes Association estimated national prevalence of 8.7%,[9] 10.6% in the San Antonio Heart Study,[6] and 14.26% in the ADS. Table 3 shows that the value of PPV increases as disease becomes more prevalent in the sample. For estimates of prevalence used to determine PPV and NPV for prediabetes/diabetes screening test, only the 45.82% prevalence found by this study (prediabetes plus diabetes) is used.

The ROC curve is used to illustrate the ability of a screening test to discriminate between diseased and normal cases. The AUC is a quantitative measure of probability of the test to make this determination. The AUC of the diabetes ROC curve was .960±.016 ($P=.0001$). The AUC of the curve for prediabetes or diabetes was .880±.016 ($P=.0001$).

Urinary TGF-β_1 was more than fivefold higher in poorly controlled versus controlled or normal subjects and had a significant positive correlation with HgbA1C (Figure 1A and 1B).

DISCUSSION

Prevalence of undiagnosed diabetes detected in the ADS was higher than expected. The estimated national prevalence of diabetes as reported by the American Diabetes Association is 8.7%,[10] 1.64 times lower than in the ADS. Because the ADS sample is predominantly Hispanic, this appears to be due to a combination of obesity[11,12] and genetic factors.[1,4] The San Antonio Heart Study, conducted in a predominantly Mexican American sample, found a similar prevalence.[13]

Sensitivity and specificity of one-hour OGTT are within the acceptable measurements to screen for either prediabetes or diabetes. ROC analysis confirms the discrimination of one-hour OGTT as excellent. The one-hour OGTT was useful for this population-based screening. The FBG is generally preferred in clinical settings, because two-hour OGTT is more expensive, time-consuming, and inconvenient.[2,14] The one-hour OGTT has desired utility because it detects impaired glucose tolerance in individuals after glucose consumption.[8,15] FBG may not represent physiologic stresses on patients' systems postprandially; two-hour and one-hour OGTT reflect the body's response to glucose. Although not a new idea,[16] one-hour OGTT can screen for high-risk individuals.

The American Diabetes Association does not recommend at-large community screening for diabetes,[10,17] suggesting targeted opportunistic screening in groups with higher prevalence of risk factors but not population-based screening. In a population at high risk, the number needed to screen, on the basis of these criteria would be a large percentage of the population. Percentages of known risk factors[10] in the ADS population were 77.95% Hispanic or Black, 41.06% age ≥45, and 92.21% with BMI ≥25 kg/m^2. Although prevalence of metabolic syndrome was not determined in the ADS sample, the San

Table 2. Demographic characteristics by adjusted normal (AN), at risk (AR), prediabetes (PD), and diabetes mellitus (DM) categories, Atascosa Diabetes Study

Characteristic	*n*	%*	AN	%†	AR	%†	PD	%†	DM	%†
Total	526	100.0	243	46.2	42	7.9	166	31.5	75	14.2
Sex										
Male	142	27.0	61	42.9	13	9.1	47	33.1	21	14.8
Female	384	73.0	182	47.4	29	7.5	119	30.9	54	14.0
Ethnicity‡										
Hispanic	406	77.2	189	46.5	35	8.6	131	32.2	51	12.6
White	116	22.0	50	43.1	7	6.0	35	30.1	24	20.7
Black	4	0.8	4	100.0	0	0.0	0	0.0	0	0.0
Age, years										
<20	16	3.0	11	68.7	0	0.0	2	12.5	3	18.7
20–29	92	17.5	59	64.1	7	7.6	20	21.7	6	6.5
30–39	137	26.1	69	50.3	11	8.1	41	29.9	16	11.7
40–49	132	25.1	58	43.9	11	8.3	41	31.1	22	16.7
50–59	90	17.1	29	32.2	12	13.3	33	36.6	16	17.8
≥60	59	11.2	17	28.8	1	1.7	29	49.1	12	20.3
BMI, kg/m^2§										
Underweight (< 18.5)	4	.8	3	75.0	0	0	0	0	1	25.0
Normal (18.5–24.9)	35	6.7	25	71.4	1	2.9	7	20.0	2	5.7
Overweight (25.0–29.9)	117	22.2	63	53.8	9	7.7	38	32.5	7	5.9
Obese I (30.0–34.9)	151	28.7	77	50.9	6	3.9	48	31.8	20	13.2
Obese II (35.0–39.9)	105	19.9	40	38.1	18	17.1	30	28.6	17	16.1
Extreme Obesity (≥ 40)	112	21.3	34	30.3	8	7.1	42	37.5	28	25.0
Uncalculable	2	.4	1	50.0	0	0	1	50.0	0	0

* Percentage of total sample (*N*=526).
† Percentage of number listed under *n* for this category.
‡ Ethnicity was determined from the patients' charts and was recorded based on self-reported information.
§ Ranges for body mass index as classified by the US National Institutes of Health. http://www.nhlbi.nih.gov/guidelines/obesity/ob_gdlns.pdf

Table 3. PPV and NPV for the one-hour PPG screening test in samples of different disease prevalence shown as percentages. The bold upper numbers represent the PPV and the lower numbers represent the NPV

Glucose Concentration (mg/dL)	PD-DM Prevalence*	DM Prevalence†		
	45.8%	**8.7%**	**10.6%**	**14.26%**
158	**79.7**	–	–	–
	81.2			
160	**79.8**	–	–	–
	79.9			
209	–	**49.2**	**54.7**	**62.9**
		98.8	98.5	97.9
210	–	**49.1**	**54.6**	**62.7**
		98.5	98.1	97.4

PPV = positive predictive value, NPV = negative predictive value, PPG = postprandial glucose, PD = prediabetes, DM = diabetes mellitus.
* Calculation of PPV and NPV for PD-DM screening test is based on the 45.82% prevalence found by this study. PD-DM prevalence is the sum of PD prevalence and DM prevalence.
† PPV and NPV of the one-hour PPG to DM test are calculated for three prevalences: the American Diabetes Association-estimated national prevalence of 8.7%, 10.6% in the San Antonio Heart Study, and 14.26% in the Atascosa Diabetes Study.

Antonio Heart Study reported prevalences of metabolic syndrome in Whites of 28.0% and in Mexican Americans of 41.4%,[18] values even higher than the 23.8% for Whites and 31.9% for Mexican-Americans from The Third National Health and Nutrition Examination Survey (NHANES III).[19] Metabolic syndrome predicts diabetes independently of other factors.[13] Thus, high prevalences of risk factors suggest that most of the population served by the AHC has at least one of these four risk factors. NHANES III showed risk of undiagnosed diabetes in Mexican American populations as double that for non-Hispanic Whites; risk of impaired OGTT is also higher among Mexican-Americans.[20] Atascosa County's population is 58.6% Hispanic, according to the US Census Bureau. Because the potential impact of diabetes is severe,

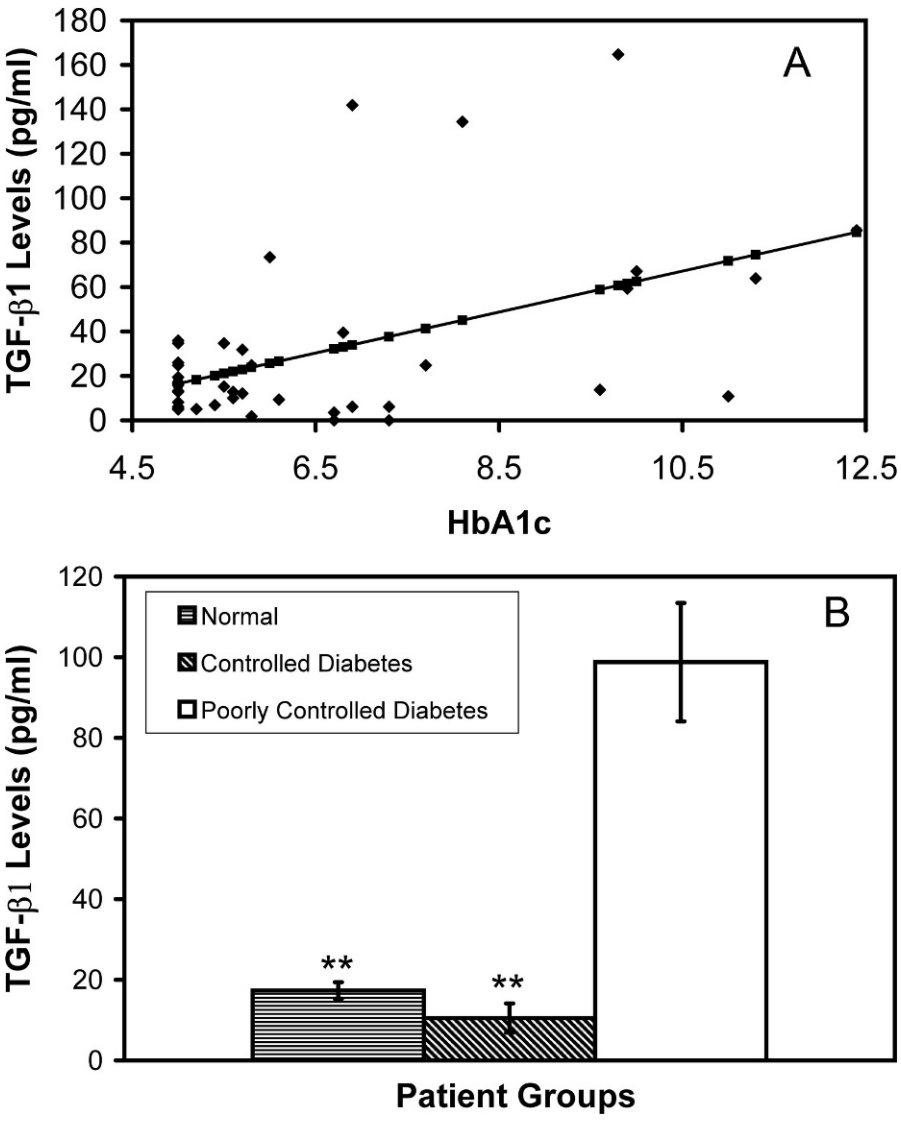

Fig 1A and 1B. Urinary transforming growth factor-β_1 (TGF-β_1) levels were correlated with hemoglobin A1C and were increased in poorly controlled diabetic patients. A) Urinary TGF-β_1 levels have a significant correlation with blood hemoglobin A1C concentrations ($P<.001$, $r^2=.24$). B) Patients with poorly controlled diabetes have urinary levels of TGF-β_1 significantly greater than normoglycemic or controlled diabetic patients, $^{**}P<.001$. Analysis of variance was significant between columns ($F[2,44]=59.66$, $P<.001$). Data are shown as means plus or minus standard error of the mean.

this study suggests that screening asymptomatic patients in Atascosa County would be prudent.

More than 45% of new end-stage renal disease (ESRD) cases in the United States are due to diabetes, and >85% of these patients have diabetes.[4] Patients with diabetic nephropathy have a markedly increased death rate from kidney failure, and the impact of diabetic nephropathy in minorities is even more pronounced. The US Renal Data System demonstrated a dramatic increase in incidence of ESRD caused by diabetes.[5] This increase could not be fully explained by changes in assignment of causes of ESRD, rising diabetes prevalence, increased renal replacement therapy access, or increased survival of patients with diabetes. While between 1984 and 1996, the ESRD population with diabetes increased by 40%, initiation of treatment for ESRD due to nephropathy increased by 300%. This increase occurred despite well-publicized studies that showed that improved glucose control might slow development or progression of nephropathy.[4,5,7] Our research suggests that one-hour PPG OGTT may be an effective tool in diagnosing patients with existing or potential glycemic dysregulation in high-risk populations, while early screening for TGF-β_1 in urine can be an effective marker for early diabetic nephropathy. These two tests may help reduce eventual morbidity and mortality due to diabetic complications.

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