Raised Serum Sialic Acid Concentration in NIDDM Patients With and Without Diabetic Nephropathy

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OBJECTIVE — Raised serum sialic acid concentration is a strong predictor of cardiovascular mortality in the general white population. A progressive increase in cardiovascular morbidity and mortality takes place in relation to increasing albuminuria in NIDDM patients. Therefore, we investigated the potential association between serum sialic acid and micro- and macroangiopathy in NIDDM patients.

RESEARCH DESIGN AND METHODS — We studied a prevalence cohort of all white NIDDM patients <76 years of age attending a diabetic clinic during 1 year. Of the patients, 319 had normoalbuminuria, 148 had microalbuminuria, and 75 had macroalbuminuria (diabetic nephropathy was in 47 of 75 patients); 66 nondiabetic age- and sex-matched subjects acted as a control group. Blood samples were taken for measurements of sialic acid, lipids, creatinine, and HbA_{1c}. Retinopathy was assessed by funduscopy. The prevalence of cardiovascular disease was based on Minnesota-coded electrocardiograms and the World Health Organization cardiovascular questionnaire.

RESULTS — A progressive raise in serum sialic acid was demonstrated with an increasing urinary albumin excretion rate: [median (range)] 2.02 (1.55–2.63); 2.42 (1.47–6.48); 2.67 (1.57–5.86), and 2.95 (1.52–7.86) mmol/l in nondiabetic subjects, NIDDM patients with normoalbuminuria, microalbuminuria, and diabetic nephropathy, respectively ($P < 0.05$ or less for differences between groups). Multiple linear regression analysis showed that serum cholesterol concentration, serum HDL cholesterol concentration, BMI, albuminuria, smoking, and cardiovascular disease correlate independently with logarithmic (10) serum sialic acid concentration.

CONCLUSIONS — Our study revealed a progressive raise in serum sialic acid with increasing urinary albumin excretion rate in NIDDM patients. Furthermore, several modifiable cardiovascular risk factors were associated with serum sialic acid.

Cardiovascular morbidity and mortality are increased in NIDDM patients, particularly if micro- or macroalbuminuria is present (1). Recently, Lindberg et al. (2) demonstrated that increased serum total sialic acid concentration is a strong predictor of cardiovascular disease (CVD) in the general white population, with raised concentrations being associated with enhanced coronary heart disease and stroke mortality (3). Several previous studies dealing with small numbers of NIDDM patients of different ethnic origin have found that serum sialic acid is elevated compared with nondiabetic subjects (4–7). However, conflicting results regarding the association between serum sialic acid and diabetic micro- and macroangiopathy have been presented (4–7).

To investigate the potential association between serum sialic acid and diabetic micro- and macroangiopathy, we studied a prevalence cohort of all white NIDDM patients <76 years of age attending a diabetic clinic during 1 year.

RESEARCH DESIGN AND METHODS

— A total of 549 NIDDM patients of European origin younger than 76 years of age were recruited from the outpatient clinic at Hvidöre Hospital during 1 year as described in detail previously (1). The patients were divided into three groups according to the level of urinary albumin excretion rate (UAER) measured by radioimmunoassay with a single antibody (8): normoalbuminuria (UAER <30 mg/24 h, $n = 323$), microalbuminuria (UAER 31–300 mg/24 h, $n = 151$), and macroalbuminuria (UAER >300 mg/24 h, $n = 75$). In all macroalbuminuric patients, 24-h urine collections were repeated, and persistent albuminuria defined as albuminuria >300 mg/24 h in at least two of three consecutive sterile nonketotic 24-h samples was documented in all 75 patients. A renal biopsy was performed in 36 of the 50 patients <66 years of age, as described previously (9). Insufficient material was obtained for one patient. All biopsies were reviewed by two observers who were unaware of the clinical features. In the remaining 40 patients, diabetic nephropathy was diagnosed clinically ($n = 20$) if the following criteria were fulfilled: persistent albuminuria <300 mg/24 h, presence of diabetic retinopathy, and no clinical or laboratory evidence of other kidney or renal tract disease other than diabetic glomerulosclerosis (9).

The control group comprising 66 individuals without diabetes (assessed by a 2-h oral glucose tolerance test) were recruited at random from a population-based epidemiological study of atherosclerotic vascular disease and its known and potential risk factors. The age and sex distributions within this control group were similar to those of the diabetic patients without macroalbuminuria. None of the control subjects had atherosclerotic vascular disease, arterial hypertension, renal disease, urinary tract infection, or in-

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CVD, cardiovascular disease; ECG, electrocardiogram; UAER, urinary albumin excretion rate.

Table 1—*Clinical data in nondiabetic control subjects and 549 NIDDM patients with normo-, micro-, or macroalbuminuria or diabetic nephropathy*

	Control subjects	Normoalbuminuria	Microalbuminuria	Macroalbuminuria	Diabetic nephropathy
n	66	323	151	28	47
Sex (M/F)	37/29	159/164	77/74†	22/6‡	38/9‖
Age (years)	55 ± 7	59 ± 12*	60 ± 10	60 ± 11	58 ± 9
BMI (kg/m^2)	24.6 ± 3.4	27.2 ± 4.8*	28.6 ± 4.6†	29.7 ± 5.1	29.5 ± 5.6
Systolic blood pressure (mmHg)	120 ± 14	150 ± 23*	162 ± 23†	158 ± 24	168 ± 24
Diastolic blood pressure (mmHg)	70 ± 9	82 ± 10*	86 ± 12†	87 ± 11§	91 ± 12‖
Duration of diabetes (years)	—	8 ± 7	10 ± 7†	8 ± 6	11 ± 6¶
CVD (%)	0	35	44†	56§	71‖
Retinopathy (%)	0	27	47†	0	72‖
HbA_{1c} (%)	5.5 (4.6–6.4)	7.9 (4.7–13.1)*	8.9 (5.3–13.5)†	8.2 (5.2–12.1)	8.6 (4.6–17.0)¶
Serum creatinine (μmol/l)	82 (34–93)	76 (29–194)	81 (38–177)	98 (55–233)‡	125 (46–740)‖
UAER (mg/24 h)	3.7 (1.0–13.8)	11 (1–30)	90 (31–261)	1,061 (52–5,108)#	1,729 (165–8,019)#
Serum cholesterol (mmol/l)	6.0 ± 0.9	6.2 ± 1.6	6.4 ± 1.5	6.4 ± 1.8	6.9 ± 1.9¶
Serum HDL cholesterol (mmol/l)	1.4 (0.7–2.8)	1.1 (0.5–3.1)	1.1 (0.4–3.1)	1.1 (0.5–3.4)	1.1 (0.5–2.1)

Continuous variables are means ± SD, or medians (range). *$P < 0.001$, normoalbuminuria vs. nondiabetic control subjects. †$P < 0.001$, micro- vs. normoalbuminuria. ‡$P < 0.05$, macro- vs. microalbuminuria. §$P < 0.05$, macro- vs. normoalbuminuria. ‖$P < 0.001$, diabetic nephropathy vs. microalbuminuria. ¶$P < 0.05$, diabetic nephropathy vs. normoalbuminuria. #Some macroalbuminuric NIDDM patients who received antihypertensive treatment had UAER <300 mg/24 h.

flammatory rheumatic disease, and none received any regular medication.

The experimental design was approved by the local ethics committee, and all patients gave their informed consent.

Clinical and laboratory measurements

All diabetic and nondiabetic participants were examined and interviewed by the same two observers (M.-A.G. and J.S.J.). We applied exactly the same clinical and laboratory measurements in the two groups. The World Health Organization cardiovascular questionnaire (10) was used to assess past and present evidence of myocardial infarction, angina pectoris, stroke, transient cerebral ischemic attack, and cardiovascular disease (CVD). Evidence of present or previous foot ulcers, present medication, and smoking habits were recorded also. Smokers were defined as persons smoking more than one cigarette/cigar/pipe per day with all others being classified as nonsmokers.

Arterial blood pressure was measured twice on the right arm after a 10-min rest in the supine position. The measurements were carried out with a Hawksley random zero sphygmomanometer (Hawksley and Sons, Lancing, Sussex, U.K.), cuff size 25 × 12 cm in lean patients and 30 × 15 cm in obese patients. Diastolic blood pressure was recorded at the disappearance of the Korotkoff sounds (phase V). Arterial hypertension was diagnosed according to the World Health Organization's criteria, systolic blood pressure ≥160 mmHg, and/or diastolic blood pressure ≥95 mmHg, or if antihypertensive treatment was being prescribed.

A 12-lead electrocardiogram (ECG) was recorded. The ECG was coded independently by two trained observers using the Minnesota codes (11). Coronary heart disease was diagnosed if the ECG showed signs of probable myocardial infarction (Minnesota code 1.1–2) or possible myocardial ischemia (Minnesota codes 1.3, 4.1, 5.1–3, or 7.1).

Ophthalmoscopy through dilated pupils was carried out by the same observer. Venous blood samples were collected in the nonfasting state and stored at −20°C.

Serum cholesterol and serum HDL cholesterol were measured with an enzymatic method (12). HbA_{1c} (normal range 4.1–6.1%) was measured by an isoelectric focusing method (13). Serum creatinine concentration was assayed by the buffered kinetic method of Jaffe (14). Serum sialic acid bound to glycoproteins and glycolipids were measured by an enzymatic colorimetric assay (15) using the sialic acid kit (Boehringer Mannheim, Mannheim, Germany). This assay is specific for sialic acid and is not influenced by glucose concentration or by frozen storage at −20°C (15).

Statistical analysis

Data on a continuous scale are given by means ± SD or medians (range) when normally or non-normally distributed, respectively. Data on a categorical scale are given by proportions. Differences between groups were sought by one-way

Table 2—*Serum sialic acid concentrations in nondiabetic control subjects and 549 NIDDM patients*

	Serum sialic acid (mmol/l)
Nondiabetic control subjects	2.02 (1.55–2.63)
NIDDM patients	
Normoalbuminuria	2.42 (1.47–6.48)*
Microalbuminuria	2.67 (1.57–5.86)†
Macroalbuminuria	3.04 (2.06–6.20)‡§
Diabetic nephropathy	2.95 (1.52–7.86)‖

Data are medians (range). Analysis of variance $P < 0.0001$. *Normoalbuminuria versus nondiabetic control subjects, $P < 0.0001$. †Microalbuminuria versus normoalbuminuria, $P < 0.0001$. ‡Macroalbuminuria versus normoalbuminuria, $P < 0.0001$. §Macroalbuminuria versus microalbuminuria, $P < 0.05$. ‖Diabetic nephropathy versus normoalbuminuria, $P < 0.0001$.

Table 3—*Simple linear regression analysis of logarithmic (10) serum sialic acid concentration and clinical and biochemical variables in 521 NIDDM patients with and without diabetic nephropathy*

Independent variables	Correlation coefficient	*P* value
Serum cholesterol	0.50	<0.0001
BMI	0.31	<0.0001
Serum HDL cholesterol	−0.26	<0.0001
Log UAER	0.22	<0.0001
HbA_{1c}	0.17	<0.0001
CVD	0.16	<0.0001
Mean blood pressure	0.13	<0.005
Smoking	0.11	<0.05
Log serum creatinine	0.10	<0.05
Sex	—	0.44
Age	—	0.85
Duration of diabetes	—	0.90

The 28 macroalbuminuric patients were excluded because of unknown causes of albuminuria.

analysis of variance and by Student's unpaired *t* test. Associations between various variables and a logarithmic (10) serum sialic acid concentration were tested by simple and multiple linear regression analysis with stepwise backward selection. Independent variables that were significantly correlated ($P < 0.05$) to serum sialic acid concentration in the simple linear regression analysis (Table 3) were included in the multiple linear regression analysis. Non-normally distributed variables were logarithmically transformed before the analyses. *P* values <5% (two-tailed) were considered to be statistically significant. The analyses were run on the personal computer statistics package SPSS for Windows version 6.0.

RESULTS— Clinical and laboratory data for the five groups studied are presented in Table 1. A progressive raise in systemic blood pressure, serum creatinine, serum cholesterol concentration, BMI, and prevalences of diabetic retinopathy and CVD occurred with increasing UAER in the NIDDM patients. Furthermore, a progressive raise in serum sialic acid with enhancing urinary albumin excretion was documented also in the NIDDM patients (Table 2). The differences between the normo-, micro-, and macroalbuminuric diabetic groups were significant ($P < 0.05$).

Simple linear regression analyses between logarithmic serum sialic acid and various clinical and biochemical variables are presented in Table 3. Multiple linear regression analysis showed serum cholesterol concentration, serum HDL cholesterol concentration, BMI, UAER, smoking, and the presence of CVD to be independently associated with logarithmic serum sialic acid concentration (Table 4). Median serum sialic acid concentrations were identical; 2.79 (1.52–6.38) vs. 2.89 (2.04–4.86) mmol/l in NIDDM patients with diabetic nephropathy and serum creatinine concentrations below (median 76 [46–93] μmol/l) or above (176 [98–740] μmol/l) the median value, respectively.

The presence or absence of diabetic retinopathy (simplex and proliferative) had no impact on serum sialic acid concentration in the different diabetic groups (Table 5). Normo- and microalbuminuric NIDDM patients suffering from CVD tended to have higher serum concentrations of sialic acid (Table 5).

CONCLUSIONS— Our cross-sectional study revealed a progressive raise in serum sialic acid concentrations with increasing UAER in NIDDM patients. Furthermore, normoalbuminuric NIDDM patients had elevated serum sialic acid concentrations compared with healthy nondiabetic control subjects, suggesting an effect per se of the diabetic state. In addition, several modifiable cardiovascular risk factors, such as serum cholesterol concentration, HDL cholesterol concentration, BMI, and smoking were associated with elevated serum sialic acid concentrations. The presence of CVD was associated with slightly higher levels of serum sialic acid in the nonproteinuric NIDDM patients. Our study revealed no independent association between kidney function as estimated with serum creatinine and serum sialic acid concentration. Furthermore, serum sialic acid concentration was nearly identical in diabetic nephropathy patients with normal or severely reduced kidney function. The presence or absence of diabetic retinopathy had no impact on the serum sialic acid levels. It should be stressed that strict criteria for diagnosing diabetic nephropathy in our patients were applied. This is crucial because ~25% of albuminuric NIDDM patients are suffering from nondiabetic glomerulopathy (9). Our patients were stratified into normo-, micro-, and macroalbuminuria according to previously published guidelines (16).

Increased serum sialic concentrations have previously been reported in 65 black and white NIDDM patients (6). No relationships were observed with the extent of CVD, diabetic nephropathy, retinopathy, blood pressure levels, and se-

Table 4—*Multiple linear regression analysis of logarithmic (10) serum sialic acid concentration with clinical and biochemical variables in 521 NIDDM patients with and without diabetic nephropathy*

Independent variable	Correlation coefficient	*P* value
Serum cholesterol	0.45	<0.0001
Serum HDL cholesterol	−0.23	<0.0001
BMI	0.20	<0.0001
Log UAER	0.19	<0.001
Smoking	0.12	<0.001
CVD	0.08	<0.05
Log serum creatinine	—	0.51
HbA_{1c}	—	0.54
Mean blood pressure	—	0.74

The 28 macroalbuminuric patients were excluded because of unknown causes of albuminuria.

Table 5—*Serum sialic acid concentration, CVD, and diabetic retinopathy in 521 NIDDM patients*

	Serum sialic acid (mmol/l)		
	Normoalbuminuric NIDDM	Microalbuminuric NIDDM	Diabetic nephropathy
CVD−	2.36 (1.47–6.48)	2.61 (1.57–5.86)	3.23 (2.23–6.38)
CVD+	2.54 (1.53–5.12)*	2.76 (1.78–4.98)	2.85 (1.52–7.86)
Retinopathy−	2.40 (1.47–5.17)	2.70 (1.57–5.86)	3.05 (2.04–6.38)
Retinopathy+	2.47 (1.63–6.48)	2.65 (1.70–5.78)	2.91 (1.52–7.86)

Data are median (range). The 28 macroalbuminuric patients were excluded because of unknown causes of albuminuria. *CVD+ vs. CVD−, $P = 0.01$.

rum cholesterol concentration. Crook et al. (4) demonstrated that total sialic acid levels were significantly elevated in a relatively small group of NIDDM patients ($n = 20$) and were correlated with hypertension and retinopathy. Tomino et al. (7) reported elevated serum sialic acid levels in 11 NIDDM patients with diabetic nephropathy compared with 15 NIDDM patients without nephropathy. Unfortunately, no information was presented regarding the criteria applied for diagnosing diabetic nephropathy. Two recent studies have demonstrated that the serum sialic acid concentration is raised in IDDM patients with both microalbuminuria and clinical proteinuria (17,18).

The mechanisms for the increased concentration of serum sialic acid in NIDDM patients with and without diabetic nephropathy are unknown, but elevated synthesis, reduced catabolism, or both must be present. Sialic acid usually occurs as a terminal component of glycoproteins and glycolipids. Consequently, we can rule out reduction in glomerular filtration as a cause of the increased serum sialic acid concentration as also demonstrated in the present study. In humans, a large quantity of sialic acid is found in the so-called acute phase reactants, i.e., orosomucoid, α_1-antitrypsin, haptoglobin, and fibrinogen (2). Our study suggests that several modifiable cardiovascular risk factors contribute to the elevation of serum sialic acid. Campos et al. (19) have demonstrated that insulin is a rapid, nonspecific, and dose-dependent (physiological dose) inhibitor of the hepatic synthesis of acute-phase proteins. Insulin deficiency and resistance are usually enhancing with an increasing degree of albuminuria in NIDDM patients. These abnormalities may act as a possible mechanism for the increasing serum sialic acid concentration. Several of the acute-phase reactants in plasma are elevated in diabetes. The progressive rise in serum sialic acid concentration with increasing albuminuria may reflect generalized vascular damage as suggested by Deckert et al. (20). Because increased serum sialic acid concentration is a strong predictor of cardiovascular mortality, Lindberg et al. (2) suggested that increased serum sialic acid concentrations may reflect the existence or the activity of an atherosclerotic process. Furthermore, serum sialic acid may reflect increased thrombogenic activity as related to the raised fibrinogen levels demonstrated in diabetes (21).

In conclusion, several modifiable cardiovascular risk factors were associated with raised serum sialic acid concentrations in NIDDM patients with and without diabetic nephropathy. Prospective studies are required to evaluate whether serum sialic acid concentration is a predictor of cardiovascular mortality in diabetes as previously demonstrated in the general population.

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