Comparative Study of Total Protein, and Total and Lipid-Associated Serum Sialic Acid Levels in Patients With Type 2 Diabetes Mellitus

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The aim of the present study was to investigate the serum total protein (TP), total sialic acid (TSA), lipid-associated sialic acid (LSA), LSA/TP, and LSA/TP values in type 2 diabetes mellitus (DM) patients. Two study groups (healthy controls and type 2 DM subjects) were examined. For the type 2 DM group, 120 patients (60 females and 60 males) who had been diagnosed and treated for type 2 DM in the Yuzuncu Yil University Hospital, Van, Turkey, were selected consecutively. Forty healthy individuals (20 females and 20 males) were selected from hospital staff and other outpatient clinics to serve as the control group. They were matched for age, sex, body mass index, and smoking status. None of

the participants had taken vitamin or mineral supplements for at least 2 weeks before sampling. To determine serum glucose, TP, TSA, and LSA levels, blood samples were drawn after all of the subjects fasted overnight. It was found that diabetics had higher TSA, LSA, TSA/TP, and LSA/TP levels than controls. However, the TP levels were not significantly different between the groups. Our results showed that TSA, LSA, TSA/TP, and LSA/TP have interactive connections with DM. These parameters can be used as a diagnostic index for patients with DM. *J. Clin. Lab. Anal.* 17:124–126, 2003.

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INTRODUCTION

Sialic acids comprise a family of acylated derivatives of neuraminic acid. They are widely distributed in mammals, and usually occur as terminal components at the non-reducing end of carbohydrate chains of glycoproteins and glycolipids (1). Elevated total serum sialic acid (TSA) concentration is a risk factor for cardiovascular mortality in humans (2). Cardiovascular disease is a common cause of death for diabetic patients (3). It has been suggested that serum TSA and lipid-associated sialic acid (LSA) levels in type 2 diabetes mellitus (DM) patients are higher than those in normal age- and sex-matched controls (4). We therefore sought to determine whether serum TSA, LSA, total protein (TP), TSA/TP, and LSA/TP have an interactive connection with diabetes, and whether they can be used as markers in diabetic patients.

MATERIALS AND METHODS

Two study groups were examined in this study. One group included 120 consecutively-selected patients who

had been newly diagnosed with type 2 DM (by fasting plasma glucose levels ≥ 126 mg/dL (7 mmol/L)) in the Yuzuncu Yil University Hospital, Van, Turkey. The second group, which served as the control, consisted of 40 healthy individuals who were selected from the hospital staff and other outpatient clinics. The controls were matched for age, sex, body mass index, and smoking status. None of the participants had taken vitamin or mineral supplements for at least 2 weeks before sampling was conducted. The characteristics of the control subjects and the type 2 DM patients are given in Table 1.

The study was conducted only after voluntary consent was obtained from the subjects. A full explanation of the study protocol was provided to the subjects before their consent was requested. The patients received care

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TABLE 1. Characteristics of diabetic patients and control subjects

	Controls (n = 40)	Diabetic patients (n = 120)
Age (year)	55.4 ± 9.454	56.8 ± 9.741
Body mass index (kg/m ²)	25.1 ± 2.2	26.33 ± 1.6
Sex (M/F)	20:20	60:60
Duration of diabetes (year)	—	6.500 ± 4.173
Serum glucose (mg/dl)	82.4 ± 9.524	181.2 ± 61.90 ^a

Values are expressed as mean ± SD.

^a*P* < 0.05 compared to control.

according to the guidelines of the World Medical Association (5).

Blood samples were drawn after the subjects fasted overnight, and were allowed to clot for serum analysis. Serum glucose was determined by routine methods using glucose oxidize, and serum TP was determined by the Biuret method on a Technicon DAX-24 (Technicon Ltd., Swords Co., Dublin, Ireland) autoanalyzer (6,7). Serum TSA concentration was determined by a method previously described by Sydow (8). Briefly, a mixture of 0.2 mL serum and 1.5 mL 5% perchloric acid (HClO₄) was incubated for 5 min at 100°C, cooled, and then centrifuged at 2,500 × *g* for 4 min. Then 0.2 mL Ehrlich's reagent was added to 1.0 mL of the clear supernatant, and heated for 15 min at 100°C. After the mixture was cooled, 1.0 mL distilled water was added to it. The optical density was measured at 525 nm in a spectrophotometer. The amount of TSA was determined by use of a standard curve developed from a standard sample of *n*-acetyl neuraminic acid.

Serum LSA concentration was determined by the method described by Katopodis et al. (9). Briefly, 44.7 µL of serum were transferred by a capillary pipette to 150 µL distilled water. The contents were vortexed for 5 sec. The tube was transferred to crushed ice. Next, a 3-mL solution of cold (4–5°C) 2:1 (v/v) chloroform : methanol was added to the tube, and the mixture was vortexed for 30 sec. To this mixture 0.5 mL cold distilled water was added, and the tube was capped. The tube was repeatedly inverted for 30 sec to mix the contents. After the tube was centrifuged for 5 min at room temperature, at 2,500 rpm, 1 mL of the upper layer was transferred into another tube. Then 50 µL of a phosphotungstic acid solution (1 g/mL) were added and mixed, and the tube was allowed to stand at room temperature for 5 min. The tube was centrifuged for 5 min at 2,500 rpm, and the supernatant was removed by suction. Then 1 mL distilled water was added and the tube was vortexed until the precipitate was in suspension with no grossly visible particles. Next, 1 mL of the resorcinol reagent was added and mixed, and the tube

was placed in boiling water for exactly 15 min. The tube was transferred to an ice-water bath and left for 10 min. To the ice-cold tube, 2 mL of 85:15 (v/v) butyl acetate:*n*-butyl alcohol were added at room temperature, and the tube was vortexed and centrifuged for 5 min at 2,500 rpm. The extracted blue color was read at 580 nm. The amount of LSA was determined by use of a standard curve developed from a standard sample of *n*-acetyl neuraminic acid.

The data were expressed as means plus standard error (SE). An unpaired *t*-test was used to compare results between the two groups. Pearson's correlation test was used to demonstrate the correlation between means in the patient group.

RESULTS

Table 2 shows the blood TP, TSA, LSA, TSA/TP, and LSA/TP levels in the diabetic patients and controls. As indicated in the table, the diabetic patients had higher (*P* < 0.05) TSA, LSA, TSA/TP, and LSA/TP levels than the controls. However, the TP levels in diabetics were not significantly different (*P* > 0.05) from those of controls.

DISCUSSION

Serum sialic acid has been termed a risk factor for cardiovascular disease in the general population (4). The causal mechanism of increased serum sialic acid concentration in diabetic patients is unknown. However, the loss of sialic acid-containing glycoproteins from vascular cells into the bloodstream, and cytokine-induced acute phase response (cytokines stimulate the hepatic production of acute phase proteins with notable sialic acid content) may lead to elevated serum sialic acid levels (10). Declining renal function can also impair the excretion of sialic acid-containing glycoconjugates and cause elevated levels of serum sialic acid (10,11).

It has been suggested that plasma TP levels decrease in rats with alloxan-induced diabetes (12). It has been also suggested that the decrease in TP is due to

TABLE 2. Blood TSA, LSA and TP levels of diabetic patients (n = 120) and controls (n = 40)

Parameters	Controls	Type 2 patients
TSA (mg/dl)	60.96 ± 6.465	70.74 ± 9.978 ^a
LSA (mg/dl)	12.55 ± 1.594	17.24 ± 2.670 ^a
TP (g/dl)	7.155 ± 0.892	6.938 ± 1.023
TSA/TP (mg/g)	8.657 ± 1.472	10.43 ± 2.186 ^a
LSA/TP (mg/g)	1.787 ± 0.355	2.542 ± 0.556 ^a

Values are expressed as mean ± SD.

^a*P* < 0.05 compared to control.

microproteinuria and albuminuria, which are important clinical markers of diabetic nephropathy, and/or to increased protein catabolism (12). However, in our study the TP levels of diabetics were no different from those of the controls. We could find nothing in the literature regarding TP levels in diabetic patients. However, we think the unchanged TP levels are due to the newly diagnosed diabetic patients used in this study.

We conclude that TSA, LSA, TSA/TP, and LSA/TP have interactive connections with DM. These parameters can be used as a diagnostic index for patients with DM.

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