Levels of Serum Sialic Acid and Thiobarbituric Acid Reactive Substances in Subjects With Impaired Glucose Tolerance and Type 2 Diabetes Mellitus

Gulsen Yilmaz,¹ Fatma Meric Yilmaz,^{1*} Yalcin Aral,² and Dogan Yucel¹

¹Clinical Biochemistry Laboratory, Ankara Education and Research Hospital, Ministry of Health, Ankara, Turkey

²Clinics of Endocrinology and Metabolism, Ankara Education and Research Hospital, Ministry of Health, Ankara, Turkey

Cardiovascular disease is a common cause of death for diabetic patients. High sialic acid levels (SA) and increased oxidative stress are important factors for cardiovascular diseases. We aimed to research whether SA and thiobarbituric acid reactive substances (TBARS) levels are associated with the degree of the diabetic regulation and investigate if SA and TBARS levels can be controlled with the regulation of the blood glucose levels. A total of 179 subjects were included in the study. Three groups, which were comprised of subjects with type 2 diabetes mellitus (DM) (DM group [DMG], $n = 149$), impaired glucose tolerance (IGT) (IGT group [IGTG], $n = 15$), and normal oral glucose tolerance (NGT) (NGTgroup [NGTG], $n = 15$) were constituted. Glucose, cholesterol, high-density lipoprotein (HDL)

and glycated hemoglobin (HbA_{1c}), SA, and TBARS were measured in the sera of the patients. SA and TBARS levels were significantly increased in subjects with type 2 DM ($P < 0.001$ for both). SA concentrations showed significant correlation with triglycerides ($r = 0.229$; $P < 0.05$), fasting glucose ($r = 0.508$; $P < 0.01$), 2-hr postprandial glucose ($r = 0.455$; $P < 0.01$), and HbA_{1c} ($r = 0.467$; $P < 0.01$), and there was a positive correlation between TBARS and HbA_{1c} ($r = 0.251$; $P < 0.01$). Diabetic patients were found to have higher risk for inflammation and oxidative stress. The regulation of blood glucose levels may contribute to the decline of both SA and TBARS levels. *J. Clin. Lab. Anal.* 21:260–264, 2007. © 2007 Wiley-Liss, Inc.

Key words: sialic acid; thiobarbituric acid reactive substances; impaired glucose tolerance; type 2 diabetes mellitus

INTRODUCTION

Sialic acid (SA) is a terminal component of the nonreducing end of carbohydrate chains of glycoproteins and glycolipids and widely occurs in the exposed positions of molecules like enzymes and hormones present in serum and tissues (1). Serum SA has been shown to be a cardiovascular risk factor and increased levels of SA were found to be associated with cardiovascular mortality as well as cerebrovascular disease (2,3). Increased levels of serum SA were also reported in patients with myocardial infarction (4,5).

Oxidative stress may play an important role in the pathogenesis of diabetes mellitus (DM). Protein glycation and glucose auto-oxidation can generate free radicals that can catalyze lipid peroxidation. Other

potential mechanisms of oxidative stress include the reduction of antioxidant defense (6–8). Malondialdehyde (MDA) is one of the well-known secondary products of lipid peroxidation after exposure to reactive oxygen species and free radicals and it may be used to evaluate oxidative damage by measuring of serum thiobarbituric acid reactive substances (TBARS) levels (9,10).

*Correspondence to: Fatma Meric Yilmaz, S.B. Ankara Eğitim ve Araştırma Hastanesi, Biyokimya Laboratuvarı, Ulucanlar Cad., Cebeci, Ankara 06340, Turkey.

E-mail: fatmamericyilmaz@hotmail.com

Received 8 February 2007; Accepted 2 May 2007

DOI 10.1002/jcla.20181

Published online in Wiley InterScience (www.interscience.wiley.com).

SA determinations in patients with DM have been made in several studies (11–13). Additional studies have reported the strong predictive value of serum SA for atherosclerosis in the general population (2,3). Therefore, serum SA may be used in the diabetic population for risk assessment of atherogenesis. Glycated hemoglobin (HbA_{1C}) is used to assess the long-term control of glucose in DM patients and tells us whether the blood glucose level has been elevated over the last 4–8 weeks. The aim of this study is to research whether SA and TBARS levels are associated with the degree of the diabetic regulation and to investigate if SA and TBARS levels can be controlled with the regulation of the blood glucose levels.

MATERIALS AND METHODS

Patients

This study included three groups, comprised of subjects with type 2 DM (DM group [DMG]), impaired glucose tolerance (IGT) (IGT group [IGTG]), and normal oral glucose tolerance (NGT) (NGT group [NGTG]). The DMG ($n = 149$) consisted of 41 men and 108 women; the mean age was 55.2 ± 15.3 years, the IGTG ($n = 15$) consisted of seven men and eight women; the mean age was 50.5 ± 15.1 years, and the NGTG ($n = 15$) consisted of seven men and eight women; the mean age was 39.5 ± 15.5 years. A total of 31 patients from the DMG had diabetic complications (11 patients had diabetic retinopathy, 15 had diabetic neuropathy, four had both retinopathy and neuropathy, and one patient had diabetic nephropathy). An oral glucose tolerance test (OGTT) with a 75-g glucose load was performed for the subjects in the IGTG and NGTG. Subjects with IGT were defined as having a 2-hr postprandial plasma glucose level between 7.8 and 11.0 mmol/L and a normal fasting plasma glucose level < 7.8 mmol/L; subjects with type 2 DM were defined as having a 2-hr postprandial plasma glucose level ≥ 11.1 mmol/L (14).

All of the patients were informed and the study was approved by the Local Ethics Committee of Ankara Education and Research Hospital.

Biochemical Analyses

Venous blood samples were obtained before breakfast following an overnight fast (12 to 14 hr) in three groups. In the IGTG and NGTG, additional blood samples were obtained after a 75-g glucose load. Serum samples were immediately separated. Glucose, cholesterol, high-density lipoprotein (HDL), and HbA_{1C} were measured on the day of sampling. For SA and TBARS measure-

ments, serum samples were stored at -20°C until the working day.

The resorcinol method was used for SA determination (15). In this method SA is first dissociated with acid hydrolysis, then resorcinol and Cu^{2+} were used to obtain chromophores. Finally, chromophores were extracted with *n*-butanol and read at 580 nm. Lipid peroxidation products generated by peroxidative processes were measured with thiobarbituric acid by the method of Hunter et al. (16) and the result was expressed as TBARS. The calibration curve was prepared with 0–25 $\mu\text{mol/L}$ malondialdehyde standards, which were generated by hydrolysis of 1,1',3,3'-tetraethoxypropane. Glucose, total cholesterol, triglycerides, and HDL cholesterol were determined in a Roche Modular D+P analyzer with the original reagents (Roche Diagnostics GmbH, Mannheim, Germany). Low-density lipoprotein (LDL) levels were calculated using the Friedewald formula (17). HbA_{1C} was determined by an Agilent 1100 HPLC analyzer (Agilent Technologies, Waldbronn, Germany), using Chromsystems reagents (Chromsystems Instruments and Chemicals GmbH, München, Germany).

Statistical Analysis

Results are presented as mean $\pm$ standard deviation (SD), with a P value less than 0.05 indicating statistical significance. Differences between the groups were compared using a nonparametric Kruskal Wallis test, and for the significant ($P < 0.05$) analytes a Mann-Whitney test was performed. Pearson correlation analysis was performed for the correlations between the variables. All statistical calculations were made using SPSS for Windows, version 13.0 (SPSS Inc., Chicago, IL).

RESULTS

SA levels were 2.43 ± 0.89 mmol/L, 1.99 ± 0.99 mmol/L, and 1.75 ± 0.15 mmol/L in DMG, IGTG, and NGTG, respectively (Fig. 1). SA levels of the DMG were significantly higher than in the IGTG and NGTG ($P < 0.001$ for both). There was not a significant difference between the IGTG and NGTG ($P = 0.061$).

TBARS levels were 6.89 ± 2.37 $\mu\text{mol/L}$, 5.5 ± 1.24 $\mu\text{mol/L}$, and 4.55 ± 0.64 $\mu\text{mol/L}$ in the DMG, IGTG, and NGTG, respectively (Fig. 1). TBARS levels of the DMG and IGTG were significantly higher than in the NGTG ($P < 0.001$ and $P < 0.01$, respectively). There was a significant difference between the IGTG and NGTG ($P = 0.029$). Other biochemical parameters were shown in Table 1. Neither SA levels nor oxidative stress parameters were significantly different in the DMG

Fig. 1. SA and MDA levels (mean $\pm$ SD) in DMG, IGTG, and NGTG (a: significantly higher than NGTG; b: significantly higher than IGTG).

TABLE 1. Biochemical parameters of the groups

	NGTG	IGTG	DMG
Fasting glucose (mmol/L)	5.21 $\pm$ 0.61	6.12 $\pm$ 0.75 ^a	9.77 $\pm$ 4.7 ^{ab}
Glucose, 2-hr postprandial (mmol/L)	5.96 $\pm$ 1.34	10.36 $\pm$ 2.39 ^a	14.35 $\pm$ 6.84 ^{ab}
HbA _{1C} (%)	5.36 $\pm$ 0.38	6.0 $\pm$ 0.08 ^a	7.70 $\pm$ 2.0 ^a
Total cholesterol (mmol/L)	4.43 $\pm$ 1.03	5.46 $\pm$ 1.02 ^a	5.20 $\pm$ 1.14 ^a
HDL cholesterol (mmol/L)	1.25 $\pm$ 0.2	1.23 $\pm$ 0.3	1.29 $\pm$ 0.32
LDL cholesterol (mmol/L)	2.41 $\pm$ 0.9	2.90 $\pm$ 0.7	2.98 $\pm$ 0.89 ^a
Triglycerides (mmol/L)	1.67 $\pm$ 0.8	2.35 $\pm$ 1.46	2.07 $\pm$ 0.99

^aSignificantly higher than NGTG.

^bSignificantly higher than IGTG.

LDL, low-density lipoprotein.

patients with diabetic complications compared with the DMG patients without diabetic complications.

We also made correlation analysis between the analytical parameters. SA showed a positive correlation with HbA_{1C} ($r = 0.467$; $P < 0.01$), fasting glucose ($r = 0.508$; $P < 0.01$), glucose 120 min ($r = 0.455$; $P < 0.01$), and triglycerides ($r = 0.229$; $P < 0.05$). TBARS showed a positive correlation with HbA_{1C} ($r = 0.251$; $P < 0.01$).

DISCUSSION

The present study focused on SA and TBARS levels in patients with IGT and type 2 DM. We found increased levels of SA and TBARS in patients with IGT and type 2 DM. Although we determined increased SA levels in both the IGTG and DMG, these elevations were statistically significant in type 2 DM patients and consistent with other studies in the literature (18,19). It has been reported that increasing serum SA concentrations were associated with atherosclerotic disease

in diabetic patients (3). This association may reflect an acute-phase response to an inflammatory process in the atherosclerotic development, or it may be the result of cytokines produced by other conditions and processes (20). Such cytokines may contribute to the development of atherosclerosis and stimulate hepatic acute phase protein production in DM (21).

Significant increase of lipid peroxidation and the role of oxidative stress in the etiology and progression of DM has been shown in animal models (6,22) and in clinical studies (7,8,23,24). It has been emphasized that nonenzymatic glycation; metabolic stress arising from the variations in the energy metabolism, sorbitol pathway activation, and hypoxia; and tissue injury resulting from ischemia-reperfusion episodes result in increased free radical production and weakened antioxidant system (25–27). In our study we showed that there was a significant increase of TBARS levels not only in the DMG but also in the IGTG. TBARS levels in the DMG were also significantly higher than in the IGTG. From this point of view elevated serum glucose levels may

increase oxidative stress and cause the higher TBARS levels and the increased oxidative stress.

Our data showed significant correlations of SA concentrations with triglycerides, fasting glucose, 2-hr postprandial glucose, and HbA_{1C}. Sialylation of lipoproteins may explain the significant correlation between SA and TG and this correlation may support the idea of accepting SA as a cardiovascular risk factor (28,29). There was a stronger correlation between SA and not only fasting glucose but also 2-hr postprandial glucose. Our study showed that there is an association between HbA_{1C} and SA. These findings support the opinion that the increase in SA levels in the diabetic population can be controlled with the strict regulation of blood glucose in diabetes.

In our study there is a positive correlation between TBARS and HbA_{1C}. There are studies indicating that free radical production is a direct result of hyperglycemia (30) and starts when endothelial and smooth muscle cells are incubated in the media with high glucose concentration (31,32).

In conclusion, we showed that SA and TBARS levels are significantly increased in subjects with type 2 DM. To our knowledge, there was not another study researching TBARS and SA levels together not only in subjects with IGT, but also in subjects with type 2 DM. SA and oxidative stress are important factors for cardiovascular diseases, which are very important components of the mortality and morbidity of diabetic patients. The regulation of blood glucose levels is shown to be important in declining both SA and TBARS levels. The use of antioxidants in IGT patients and the early period of DM may be effective in the reduction of complications of hyperglycemia by preventing oxidative stress.

REFERENCES

- Schauer R. Sialic acids and their role as biological markers. *Trends Biochem Sci* 1985;10:357–360.
- Lindberg G, Eklund G, Gullberg B, Rastam L. Serum sialic acid concentration and cardiovascular mortality. *Br Med J* 1991; 302:143–146.
- Lindberg G, Rastam L, Gullberg B, Eklund GA. Serum sialic acid concentration predicts both coronary heart disease and stroke mortality: multivariate analysis including 54,385 men and women during 20.5 years follow up. *Int J Epidemiol* 1992;21: 253–257.
- Crook M, Haq M, Haq S, Tutt P. Serum sialic acid and acute phase proteins in patients with myocardial infarction. *Angiology* 1994;45:709–715.
- Haq M, Haq S, Tutt P, Crook M. Serum total sialic acid and lipid associated sialic acid in normal subjects and those having a myocardial infarction and their relation to acute phase proteins. *Ann Clin Biochem* 1993;30:383–386.
- Pitkanen OM, Martin JM, Hallman M, Akerblom HK, Sariola H, Andersson SM. Free radical activity during development of insulin dependent diabetes mellitus in the rat. *Life Sci* 1992;50:335–339.
- Van Dam PS, Van Asbeck BS, Erkelens DW, Marx JJ, Gispen WH, Bravenboer B. The role of oxidative stress in neuropathy and other diabetic complications. *Diabetes Metab Rev* 1995;11: 181–192.
- Giugliano I, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. *Diabetes Care* 1996;19:257–267.
- Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4 hydroxynonenal, malondialdehyde and related aldehydes. *J Free Radic Biol Med* 1991;62:81–128.
- Karatas F, Karatepe M, Baysar A. Determination of free malondialdehyde in human serum by high performance liquid chromatography. *Anal Biochem* 2002;311:76–79.
- Abdella N, Akanji AO, Moijiminiyi OA, Al Assoussi A, Moussa M. Relation of serum total sialic acid concentrations with diabetic complications and cardio-vascular risk factors in Kuwaiti Type 2 diabetic patients. *Diabetes Res Clin Pract* 2000;50: 65–72.
- Tomino Y, Inoue W, Yagame M, et al. Measurement of sialic acid and acute phase reactant proteins in sera of patients with diabetic retinopathy. *J Diabet Complications* 1988;2:175–178.
- Gavella M, Lipovac V, Crnek S. Total and ganglioside bound sialic acid content of lymphocytes from non-insulin dependent diabetic patients. *Acta Diabetol Lat* 1990;27:357–364.
- World Health Organization (WHO). Definition, diagnosis and classification of diabetes mellitus and its complications. Report of a WHO consultation. Part 1: Diagnosis and classification of diabetes mellitus. Geneva: WHO; 1999. WHO/NCD/NCS/99.2.
- Svennerholm L. Quantitative estimation of sialic acids. *Biophys Acta* 1957;24:604–611.
- Hunter MIS, Nlomadim BC, Davidson DLW. Lipid peroxidation products and antioxidant proteins in plasma and cerebrospinal fluids from multiple sclerosis patients. *Neurochem Res* 1985;10: 1645–1652.
- Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18: 499–502.
- Gavella M, Lipovac V, Car A, Vučić M, Sokolić L, Rakoš R. Serum sialic acid in subjects with impaired glucose tolerance and in newly diagnosed type 2 diabetic patients. *Acta Diabetol* 2003;40: 95–100.
- Chen JW, Gall MA, Yokohama H, Jensen JS, Deckert M, Parving HP. Raised serum sialic acid concentration in DIDDM patients with and without diabetic nephropathy. *Diabetes Care* 1996;19: 130–134.
- Crook MA, Earle K, Morocutti A, Yip J, Viberti GC, Pickup JC. Serum sialic acid, a risk factor for cardiovascular disease, is increased in insulin-dependent diabetic patients with microalbuminuria and clinical proteinuria. *Diabetes Care* 1994;17:305–310.
- Campos SP, Baumann H. Insulin is a prominent modulator of the cytokine-stimulated expression of acute-phase plasma protein genes. *Mol Cell Biol* 1992;12:1789–1797.
- Bukan N, Sancak B, Yavuz Ö, et al. Lipid peroxidation and scavenging enzyme levels in the liver of streptozotocin-induced diabetes rats. *Ind J Biochem Biophys* 2003;40:447–450.
- Polak M, Zagorski Z. Lipid peroxidation in diabetic retinopathy. *Ann Univ Mariae Curie Skłodowska [Med]* 2004;59: 434–437.
- Ozdemir G, Ozden M, Maral H, Kuskay S, Cetinalp P, Tarkun I. Malondialdehyde, glutathione, glutathione peroxidase and homocysteine levels in type 2 diabetic patients with and without microalbuminuria. *Ann Clin Biochem* 2005;42(Pt 2):99–104.

25. Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. *Diabetes* 1999;48:1–9.
26. Altan N, Ongun CÖ, Hasanoğlu E, Engin A, Tuncer C, Sindel P. Effects of the sulfonylurea glyburide on superoxide dismutase activity in alloxan-induced diabetic rat hepatocytes. *Diabetes Res Clin Pract* 1994;22:95–98.
27. Elmalı E, Altan N, Bukan N. Effect of sulphonylurea glibenclamide on liver and kidney antioxidant enzymes in streptozotocin-induced diabetic rats. *Drugs R D* 2004;5:203–208.
28. Crook M, Cartwright K, Lumb P, Worsley A. Serum sialic acid in young type 1 diabetic patients. *Diabetes Res Clin Pract* 2000;47:119–122.
29. Lindberg G, Rastam L, Gullberg B, Lundblad A, Nilsson-Ehle P, Hanson BS. Serum concentrations of total sialic acid and glycoproteins in relation to coronary heart disease risk markers. *Atherosclerosis* 1993;103:123–129.
30. Giugliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. *Diabetes Care* 1996;19:257–267.
31. Rösen P, Du X, Tschöpe D. Role of oxygen derived radicals for vascular dysfunction in the diabetic heart: prevention by α -tocopherol?. *Mol Cell Biochem* 1998;188:103–111.
32. Du X, Stockklauser-Farber K, Rösen P. Generation of reactive oxygen intermediates, activation of NF Kappa B, and induction of apoptosis in human endothelial cells by glucose: role of nitric oxide synthase? *Free Radic Biol Med* 1999;27:752–763.