Relationship Between Serum Levels of Methylguanidine and Glycemic Control in IDDM Children

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OBJECTIVE— To examine the serum levels of methylguanidine in IDDM children and compare them with markers for glycemic control. Reports have indicated that active oxygen, which damages various tissues, increases in diabetes mellitus. The increase of active oxygen is one of the risk factors for diabetic complications. The synthesis of methylguanidine, a metabolic product of guanidine, is mainly regulated by active oxygen.

RESEARCH DESIGN AND METHODS— Forty-eight children with IDDM (mean age 13.3 yr) and 17 age-matched nondiabetic control subjects were studied. Diabetic children were divided into a well-controlled group (HbA_{1c} < 8%, n = 24) and a poorly controlled group (HbA_{1c} > 8%, n = 24). Serum concentrations of methylguanidine were measured by enzymatic assay.

RESULTS— Levels of methylguanidine in the poorly controlled group (1.31 ± 0.08 μM) were significantly higher than those in both the well-controlled group (0.85 ± 0.08 μM) and the control group (0.59 ± 0.11 μM), respectively (P < 0.01). Methylguanidine levels showed a positive correlation with the levels of HbA_{1c} (P < 0.01) or fructosamine (P < 0.01). No significant correlations were noted between methylguanidine levels and age, sex, duration of diabetes, or insulin dose.

CONCLUSIONS— Our data indicate that the levels of methylguanidine in IDDM children might be affected by glycemic control and that the determination of serum methylguanidine levels could be a useful test for evaluating the state of diabetic control.

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MG, METHYLGUANIDINE; IDDM, INSULIN-DEPENDENT DIABETES MELLITUS; MGAH, METHYLGUANIDINE AMIDINOHYDROLASE; MAOD, METHYLAMINE OXIDASE; MAC, METHYL-3-AMINOCROTONATE; HPLC, HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY; SOD, SUPEROXIDE DISMUTASE.

Several authors have reported that active oxygen levels increased in diabetic subjects and that active oxygen might play a role in the pathogenesis of diabetic complications (1). It was revealed that MG, a guanidine product positioned at the end of the guanidine cycle, is synthesized by the oxidation of creatinine, mainly regulated by active oxygen (2–4).

In this study, we evaluated serum concentrations of MG and correlations with glycemic control in children with IDDM. This study presents the possibility of a new marker for metabolic control in IDDM.

RESEARCH DESIGN AND METHODS

Subjects included 48 children with IDDM and 17 age-matched nondiabetic children as control subjects. Patients with continuous albuminuria, hypertension, or severe diabetic retinopathy were omitted from the study. According to HbA_{1c} levels, the diabetic children were divided into a well-controlled group (group 1: HbA_{1c} < 8%, n = 24) and a poorly controlled group (group 2: HbA_{1c} > 8%, n = 24). Three children in each group presented pubertal stage 5. Fasting morning blood samples were obtained from all subjects before insulin injection. Sera were immediately isolated and frozen at −20°C until assay. All patients received injections of intermediate and regular insulin at least twice a day. Informed consent was obtained from the patients or the parents before blood was sampled.

Serum levels of MG were examined by Nakajima et al.'s (5) method. MGAH (30 kU/g) and MAOD (0.92 kU/g) were obtained from Dr. Motoo Nakajima (Kikkoman Co., Noda, Japan). MGAH has a high order of specificity for MG and no cross-reactivity for hydrolyzed guanidino compounds with the carboxy group (5–7). For assay, enzyme reagents 1 and 2 were prepared. Enzyme reagent 1 was a Tris-HCl buffer solution (10 mM, pH 7.5) containing 14 kU/L of

Table 1—*Characteristics and laboratory data of patients and control subjects*

Group	n	(M/F)	Age (yr)	Duration of diabetes (yr)	HbA_{1c} (%)	Fructosamine (μM)	Glucose (mM)
1	24	(12/12)	13.3 ± 2.3	5.8 ± 3.1	6.8 ± 0.7*†	376 ± 11‡	9.6 ± 1.1†‡
2	24	(11/13)	13.4 ± 2.8	6.5 ± 3.2	9.2 ± 1.3‡	482 ± 16‡	13.5 ± 1.1‡
Control subjects	17	(10/7)	12.6 ± 2.1	—	3.6 ± 0.6	220 ± 11	4.9 ± 0.1

Data are means ± SE.
*$P < 0.05$ vs. control subjects.
†$P < 0.01$ vs. group 2.
‡$P < 0.01$ vs. control subjects.

MGAH and 4 kU/L of MAOD. Enzyme reagent 2 was a Tris-HCl buffer solution (10 mM, pH 7.5) containing 4 kU/L of MAOD. MAC solution was prepared by dissolving 0.2 M of MAC in DMSO.

To 1.0-ml samples in test tubes, 2.0 ml of 7.5% trichloroacetic acid was added and kept in ice-cold water for 5 min. After centrifugation (1000 *g* for 15 min), 0.5 ml of supernatant was placed into duplicate test tubes, and 0.5 ml of a carbonate buffer solution (0.5 M, pH 10.2) and 0.1 ml of enzymic reagent 1 were added to one tube (for MG determination); enzymic reagent 2 was added to the other tube (for sample blank). Each tube was incubated at 37°C for 15 min, and 0.5 ml of phosphate buffer solution (0.5 M, pH 5.5) and 0.1 ml of MAC reagent were added successively. After mixing, each tube was incubated at 37°C for 15 min. The tubes were cooled in running water for 1 min, and the fluorescences were measured at an excitation wavelength of 375 nm and an emission wavelength of 465 nm vs. the sample blank. MG levels were calculated from a standard curve. In this method, the mean recovery rate of MG was 95.1% and the intra- and interassay coefficient values were 6.1 and 4.8%, respectively. A good correlation was observed ($r = 0.974$, $n = 40$) between the levels of MG measured by the enzymic method and those measured by HPLC (5).

Blood levels of HbA_{1c}, fructosamine, and blood glucose were measured by HPLC, an enzymatic assay, and a glucose oxidase method, respectively.

Statistical analysis

Statistical analyses were performed by regression analysis and the Cochran–Cox test. All data are presented as means ± SE.

RESULTS — Table 1 presents the clinical characteristics and laboratory findings for each group. The duration of diabetes, used insulin, dose, and age were not different between the two groups of IDDM patients. HbA_{1c}, blood glucose, and fructosamine levels were significantly higher in the poorly controlled diabetic group.

MG levels in group 2 were 1.31 ± 0.08 μM, significantly higher than those of group 1 (0.85 ± 0.08 μM, $P < 0.01$) and control subjects (0.59 ± 0.11 μM, $P < 0.01$). MG levels had a significant positive correlation with HbA_{1c} ($P < 0.01$) and fructosamine ($P < 0.01$) (Fig. 1).

No correlations were noted between the MG levels in the diabetic group and those of blood glucose, age, sex, duration of diabetes, or insulin dose.

CONCLUSIONS — Decrease in SOD activity, caused by glycosylation of protein, has been suggested as one of the reasons for the increase in active oxygen (such as superoxide) in diabetes mellitus (8,9). SOD provides protection against tissue damage by active oxygen. The more SOD activity declines, the more active oxygen effect increases. Some reports have indicated that active oxygen accelerates atherogenesis in IDDM (10). On the other hand, the production of MG from creatinine is mainly regulated by active oxygen (2–4). MG could thus be an indirect indicator of active oxygen effect. In this investigation, the serum levels of MG in the poorly controlled group were significantly higher than those of the well-controlled group. MG levels presented a significant positive correlation with levels of HbA_{1c} or fructosamine. These results suggest that MG

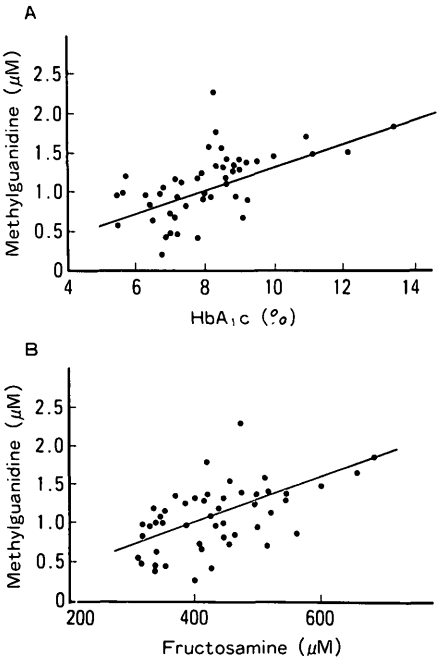

Figure 1—A: *Correlation between HbA_{1c} and MG levels* ($r = 0.58$, $P < 0.01$). B: *Correlation between fructosamine and MG levels* ($r = 0.55$, $P < 0.01$).

levels changed in relation to the state of glycemic control. A positive correlation with fructosamine suggests that the change in MG level may occur in a relatively short period of time. The reports that the activity of SOD is influenced by the level of blood glucose (9) and that significant negative correlation exists between the levels of SOD and HbA_{1c} or fructosamine in IDDM (11) are compatible with our observations.

It is difficult to determine the origin of serum MG, because various organs and tissues produce MG. The clearance rate of MG decreases in inverse proportion to the creatinine clearance (12). Because no patients subjected to this study showed high serum creatinine levels (data not shown), the high MG levels in the poorly controlled group were considered to mainly reflect the increment of MG production.

It is generally accepted that glycemic control is the most important factor for the prevention of onset or progress of diabetic complications. Our data indicate the importance of glycemic control for prohibition of serum MG increase in IDDM. Although further observation over a long period will be needed to define the relationship between MG levels and diabetic complications, our data present the possibility that serum MG could be one of the clinical markers for control of diabetes.

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