Elevated Levels of Interleukin-18 and Tumor Necrosis Factor-α in Serum of Patients With Type 2 Diabetes Mellitus: Relationship With Diabetic Nephropathy

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To compare levels of interleukin (IL)-18, tumor necrosis factor-alpha (TNF-α), and IL-6 in serum, we studied 151 type 2 diabetes mellitus patients with various degrees of nephropathy, as well as 80 healthy volunteers. IL-18, TNF-α, and IL-6 in serum were measured using an enzyme-linked immunosorbent assay (ELISA) with the respective mouse monoclonal antibodies. Significant differences in serum levels of IL-18 and TNF-α were observed between the patients and control subjects (IL-18, 278.0 ± 11.9 pg/mL *v* 172.8 ± 7.7 pg/mL, *P* < .0001; TNF-α, 2.41 ± 0.18 pg/mL *v* 0.46 ± 0.18 pg/mL, *P* < .0001), whereas that of IL-6 was not different between the two groups (0.73 ± 0.10 pg/mL *v* 0.65 ± 0.08 pg/mL, difference not significant [NS]), although patients with nephropathy showed higher levels. In addition, IL-18 levels were increased in diabetic patients with the development of urinary albumin excretion, with the highest found in those with microalbuminuria (<30 μg/mg creatinine, 252.7 ± 16.4 pg/mL; 30 to 300 μg/mg creatinine, 352.7 ± 35.2 pg/mL; >300 μg/mg creatinine, 350.0 ± 16.0 pg/mL). Similarly, TNF-α and IL-6 in diabetic patients with microalbuminuria or clinical albuminuria were significantly increased as compared with those without albuminuria (TNF-α, 3.20 ± 0.41 pg/mL *v* 1.94 ± 0.18 pg/mL; IL-6, 1.64 ± 1.11 pg/mL *v* 0.51 ± 0.05 pg/mL, *P* < .05, respectively). These results suggest that serum levels of IL-18, TNF-α, and IL-6 may have some etiopathogenic roles in diabetic nephropathy.

SEVERAL STUDIES have reported on the serum concentrations of proinflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor-alpha (TNF-α) in type 2 diabetes mellitus patients,[1-3] while increased serum concentrations of IL-6 have also been found.[1] However, the level of TNF-α in these patients remains controversial.[4-6]

IL-18, an interferon-γ–inducing proinflammatory cytokine, is known to be involved in various disease states; however, to the best of our knowledge, very little has been reported about its involvement in diabetes mellitus. Moreover, the pathogenesis of diabetic nephropathy is not completely understood, despite extensive investigations. Therefore, we measured the concentrations of IL-18, together with TNF-α and IL-6, in serum samples from type 2 diabetic patients to elucidate the etiopathological role, if any, of these cytokines in diabetes mellitus as well as diabetic nephropathy.

MATERIALS AND METHODS

One hundred fifty-one patients (61 males and 90 females) with type 2 diabetes mellitus, recruited from our outpatients, together with 80 control subjects (48 males and 32 females) who had applied for an annual health examination, were enrolled in the present study after informed consent was obtained from each. All patients and control subjects were aged 65 years or younger. Control subjects were judged normal after a physical examination, as well as standard hematological, biochemical, and radiological evaluations. Diagnosis of type 2 diabetes mellitus was made based on the criteria of the American Diabetes Association.[7] Fifty-seven of the patients were receiving insulin and 66 were receiving oral hypoglycemic agents, while 28 were on diet therapy alone. Patients with acute illness or taking drugs that might have had some effects on serum cytokine levels were excluded. Albumin excretions in spot urine samples, collected during outpatient clinic examinations, were estimated on at least 2 separate occasions and the patients were classified into 3 groups according to the definition of abnormalities in albumin excretion advocated by the American Diabetes Association,[8] with less than 30 μg/mg creatinine, 30 to 300 μg/mg creatinine, and greater than 300 μg/mg creatinine used to represent normal, microalbuminuria, and clinical albuminuria, respectively.

Serum levels of IL-18, TNF-α, and IL-6 were measured using an enzyme-linked immunosorbent assay (ELISA) with the respective mouse monoclonal antibodies, while concentrations of IL-18 in serum were measured with a Human IL-18 ELISA Kit (Medical & Biological Laboratories, Nagoya, Japan), with a minimum detectable concentration of 12.5 pg/mL. The intra-assay coefficients of variation (CVs) of IL-18 were between 4.9% (at 601 pg/mL) and 9.9% (at 69.7 pg/mL), and the interassay CVs were from 5.2% (at 615.1 pg/mL) to 10.1% (at 2621.1 pg/mL). Serum concentrations of TNF-α and IL-6 were measured with a Human TNF-α ELISA Kit and Human IL-6US ELISA Kit, respectively (Biosource International, Camarillo, CA). The minimum detectable concentrations of TNF-α and IL-6 were 0.09 pg/mL and 0.104 pg/mL, respectively. The intra-assay CVs of TNF-α were between 3.9% (at 459 pg/mL) and 5.2% (at 58 pg/mL), and the interassay CVs were from 5.9% (at 438 pg/mL) to 8.5% (at 47 pg/mL), while those of IL-6 were between 4.7% (at 9.19 pg/mL) and 8.3% (at 1.92 pg/mL), and 6.7% (at 8.81 pg/mL) and 10.0% (at 2.0 pg/mL), respectively.

When TNF-α and IL-6 levels were less than 0.09 and 0.104 pg/mL, respectively, those values were used for statistical analyses. C-peptide was measured by an enzyme immunoassay. Data are reported as means ± SE, unless otherwise specified. Observed differences between and among groups were compared by analysis of variance (ANOVA). Correlations between 2 variables were estimated by Spearman's rank sum test. A *P* value less than .05 was considered statistically significant.

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Table 1. Serum Levels of IL-18, TNF-α, and IL-6, Along With Demographic Data of the Patients and Control Subjects

	Total			Male			Female		
	DM (n = 151)	Control (n = 80)	*P* Value	DM (n = 61)	Control (n = 48)	*P* Value	DM (n = 90)	Control (n = 32)	*P* Value
IL-18 (pg/mL)	278.0 ± 11.9	172.8 ± 7.7	<.0001	305.2 ± 16.6	195.1 ± 9.9	<.0001	258.1 ± 16.4	138.1 ± 9.2	<.0001
TNF-α (pg/mL)	2.41 ± 0.18	0.46 ± 0.18	<.0001	2.72 ± 0.37	0.53 ± 0.29	<.0001	2.18 ± 0.16	0.35 ± 0.14	<.0001
IL-6 (pg/mL)	0.73 ± 0.10	0.65 ± 0.08	NS	0.75 ± 0.13	0.77 ± 0.13	NS	0.72 ± 0.15	0.45 ± 0.06	NS
Age (yr)	53.1 ± 0.9	54.8 ± 0.6	NS	53.9 ± 1.4	56.0 ± 0.6	NS	52.5 ± 1.2	52.8 ± 1.1	NS
FPG (mg/dL)	167.7 ± 4.3	95.5 ± 0.7	<.0001	169.2 ± 6.0	98.2 ± 0.9	<.0001	166.6 ± 6.1	91.5 ± 1.0	<.0001
HbA_{1c} (%)	8.2 ± 0.1	ND	—	8.3 ± 0.2	ND	–	8.2 ± 0.2	ND	–
BMI (kg/m^2)	22.9 ± 0.2	23.3 ± 0.3	NS	24.0 ± 0.2	23.4 ± 0.3	NS	22.0 ± 0.2	23.2 ± 0.6	<.05

Abbreviations: DM, diabetes mellitus; BMI, body mass index; FPG, fasting plasma glucose; HbA_{1c}, hemoglobin A_{1c}; ND, not determined; NS, not significant.

RESULTS

Clinical Characteristics of Subjects

There were no significant differences between the diabetic patients and control subjects regarding age and body mass index (BMI), but the patient group had higher fasting plasma glucose (FPG) values (Table 1). Gender composition was significantly different between the 2 groups (male/female: diabetic group *v* controls, 61/90 *v* 48/32; $P < .01$). Macroangiopathy was found in 6 patients (4 with an old cerebral infarction, 1 with an old myocardial infarction, and 1 with arteriosclerosis obliterans [ASO]), who showed higher serum levels of IL-18 and TNF-α than those without macroangiopathy (IL-18, 325.9 ± 53.7 pg/mL *v* 276.1 ± 12.2 pg/mL; TNF-α, 3.60 ± 0.58 pg/mL *v* 2.37 ± 0.19 pg/mL). However, patients with macroangiopathy showed lower serum levels of IL-6 than those without macroangiopathy (0.49 ± 0.12 pg/mL *v* 0.74 ± 0.11 pg/mL), although the differences were not statistically significant.

Demographic Profiles of Diabetic Patients According to Urinary Albumin Excretion

There were no significant differences in age, hemoglobin A_{1c} (HbA_{1c}), BMI, and C-reactive protein (CRP) among patients with and without albuminuria (Table 2).

Serum IL-18

Serum IL-18 levels were significantly higher in the patients than control subjects, and similar results were obtained when male and female patients were compared separately (Table 1). As shown in Table 2, serum IL-18 was significantly increased in the diabetic patients, even in those without microalbuminuria, when compared with the control subjects (252.7 ± 16.4 pg/mL *v* 172.8 ± 7.7 pg/mL, $P < .05$). Furthermore, patients with microalbuminuria or clinical albuminuria showed higher levels of serum IL-18 than those without microalbuminuria. However, no significant difference in IL-18 level was observed between patients with microalbuminuria and clinical albuminuria. Similar tendencies were observed when male and female diabetics with microalbuminuria were examined separately, except for 1 female patient with clinical albuminuria who showed a decreased level of serum IL-18 (141.6 pg/mL). Further, there were significant relationships between serum IL-18 levels and FPG or HbA_{1c} (IL-18 *v* FPG, $r = 0.263$, $P < .0001$; IL-18 *v* HbA_{1c}, $r = 0.188$, $P < .05$). In contrast, there was no significant relationship between serum IL-18 levels and C-peptide (data not shown).

Serum TNF-α

There was a significant difference in TNF-α serum levels between the patients and control subjects, and similar results were obtained when male and female patients were compared separately (Table 1). In addition, when patients with diabetes mellitus were divided into 3 groups according to the degree of albumin excretion, diabetic patients showed higher TNF-α levels compared with control subjects, irrespecive of the degree of albuminuria; however, patients with microalbuminuria showed higher TNF-α levels than those without microalbuminuria (Table 2). When male patients and female patients were compared separately along with the controls, similar tendencies were observed, except for between male patients with clinical albuminuria and control subjects (1.85 ± 0.52 pg/mL *v* 0.53 ± 0.29 pg/mL, difference not significant [NS]). Further, there was a significant relationship between serum TNF-α levels and FPG

Table 2. Serum Levels of IL-18, TNF-α, and IL-6, Along With Demographic Data for All Diabetic Patients According to Urinary Albumin Excretion

	Urinary Albumin Excretion		
	Normal (n = 74)	Microalbuminuria (n = 23)	Clinical Albuminuria (n = 7)
IL-18 (pg/mL)	252.7 ± 16.4	352.7 ± 35.2*	350.0 ± 16.0*
TNF-α (pg/mL)	1.94 ± 0.18	3.20 ± 0.41*	2.10 ± 0.50
IL-6 (pg/mL)	0.51 ± 0.05	0.79 ± 0.16	1.64 ± 1.11*†
Age (yr)	51.8 ± 1.3	54.7 ± 2.4	56.6 ± 2.1
BMI (kg/m^2)	22.7 ± 0.3	23.1 ± 0.4	24.2 ± 0.7
FPG (mg/dL)	167.2 ± 5.6	170.9 ± 12.5	149.4 ± 13.7
HbA_{1c} (%)	8.2 ± 0.2	8.6 ± 0.3	8.0 ± 0.4
TG (mg/dL)	93.1 ± 6.0	97.6 ± 8.2	103.6 ± 17.2
T-C (mg/dL)	214.2 ± 3.8	219.2 ± 5.7	243.6 ± 27.4*
HDL-C (mg/dL)	69.8 ± 2.4	68.1 ± 4.4	62.4 ± 11.3
CRP (mg/dL)	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.2
Cr (mg/dL)	0.60 ± 0.02	0.65 ± 0.04	0.77 ± 0.08*

NOTE. Normal, spot urine albumin excretion < 30 μg/mg creatinine; microalbuminuria, spot urine albumin excretion 30 to 300 μg/mg creatinine; clinical albuminuria, > 300 μg/mg creatinine.

Abbreviations: TG, triglyceride; T-C, total cholesterol; HDL-C, high-density lipoprotein cholesterol; CRP, C-reactive protein; Cr, creatinine.

*$P < .05$ *v* normal.

†$P < .05$ *v* microalbuminuria.

(TNF-α *v* FPG, $r = 0.263$, $P < .0001$), although no such relationships were observed between HbA_{1c}, C-peptide, age, and CRP (data not shown).

Serum IL-6

Serum IL-6 levels were not different between the patients and control subjects, and similar results were obtained when male and female patients were compared separately (Table 1). As seen in Table 2, serum IL-6 increased in an almost linear fashion in accordance with the increase in urinary albumin excretion, and diabetic patients with clinical albuminuria had levels that were significantly increased over those with and without microalbuminuria, as well as over the control subjects. Similar results were observed only in male diabetic patients (data not shown). However, there were no significant relationships between serum IL-6 levels and other biochemical parameters such as $HbA_{1c,}$ FPG, or C-peptide, although relationships were observed between age and CRP (data not shown).

DISCUSSION

A number of studies have shown that serum IL-18 levels are elevated in various immune[9-12] and nonimmune diseases.[13] However, very little is known about the relationship between diabetes mellitus and IL-18, except for several reports that have noted the possible involvement of IL-18 with type 1 diabetes mellitus.[14-16] In contrast, there is no known detailed study of IL-18 with type 2 diabetes mellitus.

Several reports have noted increased serum concentrations of IL-6 and TNF-α in type 2 diabetes mellitus patients.[1,2,5] Although our study included a greater number of female diabetic patients in whom TNF-α was not found to be increased,[6] the present results also indicated that TNF-α was increased in type 2 diabetes. In contrast, IL-6 was not found to be elevated in the present patients, even though increased serum concentrations of IL-6 have been described previously.[1] Unfortunately, the underlying cause of these discrepancies remain unclear from our data.

We considered that our most intriguing observations were as follows: (1) the serum concentration of IL-18 in type 2 diabetes patients was significantly higher than in nondiabetic control subjects; (2) the level of IL-18 in serum was correlated with glycemic control, as reflected by FPG and HbA_{1c}; and (3) serum IL-18 levels increased with the development of diabetic nephropathy.

Serum levels of IL-18 and TNF-α were increased in type 2 diabetes mellitus patients; however, the concentrations of these cytokines did not reflect inflammation, as indicated by our results, which showed no relationship with CRP. In contrast, increased serum levels of IL-18 and TNF-α may have been related to glycemic state and diabetic nephropathy. However, the correlations between serum levels of IL-18 and TNF-α and glycemic state were so weak that the biological importance of their correlations is questionable. C-peptide was also compared to IL-18, TNF-α, and IL-6 in the diabetic patients. However, there were no significant relationships between C-peptide and any of the cytokines. This result may be ascribable to the patient characteristics, since all were type 2 diabetics in which β-cell function may have been fairy well preserved. Therefore, it is difficult to explain why IL-18 or TNF-α is elevated in type 2 diabetic patients, especially in those with nephropathy. IL-18 may have some etiopathogenic role in the development of diabetic nephropathy, since it is increased with the presence of albuminuria, as is TNF-α, and it may also reflect early diabetic nephropathy, since increased levels were observed in diabetic patients without overt proteinuria.

As for the origin of IL-6, an in situ hybridization histochemical study of renal tissue from patients with diabetic nephropathy demonstrated IL-6 mRNA in glomerular resident cells.[17] Similarly, the increased serum IL-18 levels in type 2 diabetes mellitus patients found in the present study may have been derived from those tissues. Although IL-18 is constitutively expressed in renal tubular epithelia,[18] it has also been reported to be increasingly released from tubular cells during ischemic acute renal failure.[19] Thus, it is probable that increased levels of IL-18 are also released from tubular cells in diabetic states and that the cytokine plays a deleterious role in diabetic nephropathy. However, when considering the pathogenesis of diabetic nephropathy, the possibility of a glomerular origin of IL-18 cannot be fully excluded. It is well known that macrophages infiltrate glomeruli and/or interstitium in renal tissue in diabetic patients with nephropathy. Therefore, infiltrating macrophages may be responsible for increased levels of IL-18, as the highest IL-18 levels were observed in patients with microalbuminuria, in contrast to those with clinical albuminuria, in whom the infiltration of macrophages may have ceased.

In conclusion, the present study demonstrated for the first time that serum IL-18 levels were increased in type 2 diabetes mellitus patients, especially in those with nephropathy. In addition, it was shown that serum TNF-α was also increased in the same type of patients. However, the cause-effect relationships between IL-18 or TNF-α and type 2 diabetes mellitus or diabetic nephropathy remain undetermined from the present results, and additional studies will be required to understand those issues. Moreover, the relationship between IL-18 or TNF-α and advanced glycation end-product proteins should be investigated, since they have been implicated in the pathogenesis of diabetic complications.

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