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Soluble tumor necrosis factor receptor 2 is independently associated with pulse wave velocity in nonobese Japanese patients with type 2 diabetes mellitus

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Abstract

The aim of the present study was to investigate the factors contributing to pulse wave velocity (PWV) in patients with type 2 diabetes mellitus. We focused on tumor necrosis factor (TNF) including soluble TNF receptors (sTNF-R1, sTNF-R2) in this study because TNF seems to be associated with the progression of atherosclerosis and because the relationships between PWV and TNF were not yet examined in type 2 diabetic patients. Univariate regression analyses showed that PWV was positively correlated with age ($r = 0.492$, $P < .001$), diabetes duration ($r = 0.251$, $P = .021$), systolic ($r = .595$, $P < .001$) and diastolic ($r = 0.248$, $P = .022$) blood pressure, antihypertensive medication ($r = 0.268$, $P = .013$), and the concentrations of sTNF-R1 ($r = 0.354$, $P = .001$) and sTNF-R2 ($r = 0.415$, $P < .001$). Although there was a positive correlation between TNF-α and sTNF-R1 ($r = 0.382$, $P < .001$) or sTNF-R2 ($r = 0.394$, $P < .001$), TNF-α was not associated with PWV. Other variables including gender were not associated with PWV. Multiple regression analyses showed that PWV was independently predicted by the level of age (F = 15.1), systolic blood pressure (F = 31.6), and sTNF-R2 (F = 5.2), which explained 49.2% of the variability of PWV. From these results, it can be concluded that serum soluble TNF receptor is an important independent factor associated with aortic PWV in type 2 diabetic patients.

1. Introduction

Type 2 diabetes mellitus is associated with high mortality and morbidity due to atherosclerosis including coronary heart disease (CHD). As regards the risk factors responsible for the evolution of atherosclerosis in diabetic patients, Bierman [1] previously estimated that typical risk factors including blood pressure, cholesterol, and smoking can account for no more than 25% to 30% of excess cardiovascular risk factors in diabetic patients. Thus, other factors seem to play a major role in the progression of atherosclerosis in diabetes.

A number of studies have identified abnormalities of arterial stiffness in subjects with diabetes [2-4]. It has recently been reported that aortic stiffness measured by pulse wave velocity (PWV) is highly predictive of cardiovascular mortality in subjects with type 2 diabetes mellitus [5]. PWV also predicts cardiovascular mortality in nondiabetic subjects [6]. Whereas age and blood pressure are shown to be associated with PWV, age and blood pressure alone do not completely account for the abnormalities of aortic stiffness in subjects with type 2 diabetes mellitus.

Tumor necrosis factor α (TNF-α), the proinflammatory cytokine, seems to be associated with the progression of

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atherosclerosis in type 2 diabetes mellitus. There is, however, a paucity of the literature regarding the relationship between TNF-α and atherosclerosis in type 2 diabetic patients. We recently demonstrated that TNF-α system activity, especially soluble TNF-R1 (sTNF-R1), is strongly and independently associated with albuminuria in type 2 diabetic patients [7]. Klein et al [8] demonstrated that TNF-α influences the metabolism of glycosaminoglycans, which are components of the vascular endothelium and the glomerular basement membrane and are involved in the etiology of microalbuminuria. Shai et al [9] demonstrated that sTNF-R2 is strongly associated with risk of CHD in patients with type 2 diabetes mellitus. It is therefore hypothesized that there is a causal relationship between TNF-α and vascular complications in diabetic patients. To the best of our knowledge, however, the relationships between serum soluble TNF receptor and PWV have not yet been evaluated in type 2 diabetes mellitus.

In this context, a major problem is that atherosclerotic disease such as CHD, renal failure, stroke, and peripheral arterial occlusive disease of the lower extremities might affect PWV. Moreover, it is well recognized that being overweight or hyperglycemic per se might affect serum concentrations of TNF-α and soluble TNF receptor in humans [10,11]. To disclose the mechanisms responsible for the early stage of atherosclerosis, we recruited nonobese, well-controlled, unique Japanese type 2 diabetic patients who had no evidence of vascular complications including CHD, cerebral infarction, renal failure, and peripheral arterial occlusive disease, taking into account body mass index (BMI) and fasting glucose. This is the first description of serum level of soluble TNF receptor being independently associated with PWV in nonobese, well-controlled, unique Japanese type 2 diabetic patients.

2. Subjects and methods

Eighty-six Japanese type 2 diabetic patients who visited Kansai-Denryoku Hospital were enrolled for the present study. They had no abnormal electrocardiogram findings suggestive of ischemic heart disease. They also had normal serum creatinine level (<1.0 mg/dL), ankle brachial index greater than 1.0, and no signs of cerebral stroke. Thus, they were considered to have no major cardiovascular disease at the time of the study. Type 2 diabetes mellitus was diagnosed based on the criteria of the World Health Organization [12]. The subjects had no evidence of current acute illness or infectious process. The duration of diabetes was 11.1 ± 0.8 years (mean ± SEM). Seventy-five of 86 patients were taking sulfonylureas (gliclazide) and the rest were controlled with diet alone. None had received insulin therapy or any medications known to enhance insulin sensitivity such as biguanide or pioglitazone. Before the study, 2 dietitians confirmed, by checking daily food records, that the subjects had ingested at least 150 g of carbohydrate for the 3 days preceding the study. Blood pressure was measured twice with Colin BP-103iII (Tokyo, Japan) while the patients were in the sitting position after at least 5 minutes rest and the mean value was used for the analysis. Hypertension was defined as systolic blood pressure of 140 nbsp;mm Hg or higher and/or diastolic blood pressure of 90 mm Hg or higher or current use of antihypertensive medication. On the day of the examination, they were told not to take all medications including sulfonylurea, antihypertensive medications, and lipid-lowering agents. Thirty-four (40%) patients were treated with antihypertensive medications. Twenty-nine (34%) of 86 patients were receiving lipid-lowering agents (bezafibrate, 17; HMG-CoA reductase inhibitor, 12). Cigarette smoking was dichotomized into "never" and "ever" (including past and current) by use of a questionnaire. They were told not to smoke at least 1 day before the study. They did not consume alcohol or perform heavy exercise for at least 1 week before the study.

Blood was drawn in the morning after a 12-hour fast. Plasma glucose was measured with the glucose oxidase method. Triglyceride, total cholesterol, and high-density lipoprotein (HDL) cholesterol levels were also measured. The low-density lipoprotein (LDL) cholesterol level was calculated with the Friedewald formula [13]. Serum insulin was measured by a two-site immunoradiometric assay (Insulin Riabead II, Dainabot, Osaka City, Japan). Coefficients of variation were 4% and 7% for insulin greater than and insulin less than 25 μU/mL, respectively.

The estimate of insulin resistance by homeostasis model assessment (HOMA-IR) was calculated with the formula: fasting serum insulin (μU/mL) × fasting plasma glucose (mmol/L)/22.5 [14]. HOMA-IR was validated in diabetic subjects with diet therapy alone and in those treated with sulfonylureas [15,16]. HOMA-IR greater than 2.5 was defined as insulin resistance [17,18]. Serum TNF-α concentrations were measured by an enzyme immunoassay kit (Quantikine HS Human TNF-α immunoassay kit, R&D Systems, Minneapolis, MN), and serum concentrations of sTNF-R1 and sTNF-R2 were measured by enzyme-linked immunosorbent assay (BIOTRAK, Amersham Life Sciences, Uppsala, Sweden) as described previously [7,19]. The limits of sensitivity for TNF-α, sTNF-R1, and sTNF-R2 were 0.5, 25, and 50 pg/mL, respectively. The intra-assay coefficients of variation for TNF-α, sTNF-R1, and sTNF-R2 were 5.9%, 4.7%, and 3.2%, respectively. The inter-assay coefficients of variation for TNF-α, sTNF-R1, and sTNF-R2 were 10.8%, 5.8%, and 3.6%, respectively.

2.1. Measurement of PWV

Pulse wave velocity was measured with a volume plethysmographic apparatus (form PWV/ABI version-112, Colin, Komaki, Japan). Briefly, after an overnight fast, the subjects were examined in the early morning while in the supine position. Electrocardiogram electrodes were placed on both wrists. A microphone for detecting heart sounds was placed on the left edge of the sternum. The cuffs were wrapped on both the brachia and ankles. The characteristic

Table 1
Clinical profiles of patients studied

	All patients	Male patients	Female patients
n	86	61	25
Age (y)	62.8 ± 1.0	61.3 ± 1.1	66.3 ± 1.5*
Duration of diabetes (y)	11.1 ± 0.8	11.1 ± 1.0	11.2 ± 1.3
Smoking (%)	26	33	8**
BMI (kg/m^2)	22.8 ± 0.3	23.1 ± 0.2	22.2 ± 0.6*
Systolic blood pressure (mm Hg)	136 ± 2	134 ± 2	140 ± 4
Diastolic blood pressure (mm Hg)	82 ± 1	83 ± 1	81 ± 2
Sulfonylurea/diet	76/10	55/6	21/4
HMG-CoA reductase inhibitor (%)	15	11	24*
Bezafibrate (%)	21	21	20
Antihypertensive agent (%)	42	36	56
Fasting glucose (mg/dL)	142 ± 3	144 ± 3	133 ± 5
HbA_{1c} (%)	7.0 ± 0.1	7.0 ± 0.1	7.1 ± 0.2
Fasting insulin (μU/mL)	6.6 ± 0.4	6.7 ± 0.4	6.3 ± 0.7
HOMA-IR	2.32 ± 0.15	2.40 ± 0.17	2.13 ± 0.24
Triglyceride (mg/dL)	122 ± 6	128 ± 8	105 ± 9*
HDL cholesterol (mg/dL)	59 ± 2	55 ± 2	66 ± 3**
Total cholesterol (mg/dL)	204 ± 4	201 ± 4	210 ± 9
LDL cholesterol (mg/dL)	126 ± 4	126 ± 4	128 ± 8
Serum creatinine (mg/dL)	0.76 ± 0.02	0.82 ± 0.02	0.63 ± 0.04**
Serum urea nitrogen (mg/dL)	15.3 ± 0.4	15.2 ± 0.4	15.6 ± 0.9
TNF-α (pg/mL)	3.3 ± 0.2	3.60 ± 0.29	2.65 ± 0.19*
sTNF-R1 (pg/mL)	1184 ± 44	1184 ± 41	1185 ± 100
sTNF-R2 (pg/mL)	2053 ± 57	2070 ± 59	2013 ± 115
PWV (cm/s)	1661 ± 34	1663 ± 37	1655 ± 59

* $P < .05$ vs male patients.
** $P < .01$ vs male patients.

points of wave forms were determined automatically and the results were printed out. All procedures took about 5 minutes. The interobserver and intraobserver variation coefficients were 8.4% and 10.0%, respectively. Measurements on different days revealed that slight changes in blood pressure did not correlate with changes in PWV. The mean PWV value measured on either side of each patient was used for the analysis.

2.2. Statistical analysis

Data were presented as mean ± SEM. Statistical analyses were conducted with the StatView 5 system (Statview, Berkeley, CA). Simple (Spearman rank) correlation coefficients between PWV and measures of variables were calculated, and a stepwise multiple regression analysis was then used to evaluate the independent association of these variables with PWV. The means of the 2 groups (male vs female patients) were compared with Student t test. $P < .05$ was considered as significant. In multivariate analysis, an F value of 4 or greater was considered significant.

3. Results

Clinical characteristics of all subjects are summarized in Table 1. They were all Japanese type 2 diabetic patients (61 men and 25 women) with an age range of 43 to 84 years and a BMI of 17.1 to 26.7 kg/m^2. They all were nonobese [20]. The ranges of fasting glucose and glycosylated hemoglobin (HbA_{1c}) were from 92 to 194 mg/dL and from 4.9% to 10.1%, respectively. There was a wide variation in insulin resistance calculated from HOMA-IR (range, 0.51-7.17). Thirty-one (36%) of the 86 subjects had HOMA-IR greater than 2.5, indicating that they were insulin resistant [17,18].

Clinical features of male and female patients are shown in Table 1. Although a significant difference was observed in age, smoking status, BMI, triglycerides, HDL cholesterol, creatinine, and TNF-α between the 2 groups, there was no significant difference in some variables including sTNF-R1, sTNF-R2, and PWV between the 2 groups.

Values of PWV ranged from 1139 to 2728 cm/s (mean, 1661 cm/s; SD, 292 cm/s) (Table 1). Only 17 (20%) of 86 patients had PWV less than 1400 cm/s (range, 1139-1385 cm/s). This finding was far different from the recent report by Kim et al [21] in which 90% of the PWV values were between 525 and 1399 cm/s in 2488 healthy individuals. We therefore considered all patients as a group and investigated the relationships between PWV and some variables including TNF-α with univariate and multiple regression analyses.

Table 2
Correlation of brachial-ankle PWV with measures for variables in all diabetic patients

	Univariate		Multivariate
	r	P	F
Age	0.492	<.001	15.1
Diabetes duration	0.251	.021	2.9
Systolic blood pressure	0.595	<.001	31.6
Diastolic blood pressure	0.248	.022	0.4
TNF-α	0.167	.123	–
sTNF-R1	0.354	.001	0.1
sTNF-R2	0.415	<.001	5.2
Gender	−0.032	.765	–
Smoking	−0.047	.663	–
BMI	−0.113	.296	–
Fasting glucose	0.075	.492	–
HbA_{1c}	0.054	.620	–
Insulin	−0.007	.950	–
HOMA-IR	0.019	.861	–
Triglycerides	−0.095	.382	–
Total cholesterol	−0.035	.747	–
HDL cholesterol	0.036	.743	–
LDL cholesterol	−0.043	.692	–
Serum creatinine	0.079	.465	–
Therapy for diabetes	−0.023	.831	–
Therapy for hypertension	0.268	.013	2.1
Therapy for triglyceride	0.055	.610	–
Therapy for cholesterol	0.016	.881	–

Table 2 illustrates the correlation between PWV and the measures of variables including age, sex, and TNF in all diabetic patients. PWV was positively correlated with age ($r = 0.492$, $P < .001$), diabetes duration ($r = 0.251$, $P = .021$), systolic blood pressure ($r = 0.595$, $P < .001$), diastolic blood pressure ($r = 0.248$, $P = .022$), sTNF-R1 ($r = 0.354$, $P = .001$), and sTNF-R2 ($r = 0.415$, $P < .001$). The difference in PWV was also observed between the patients taking antihypertensive medications and those who were not ($r = 0.268$, $P = .013$). However, other variables including TNF-α, sex, smoking status, BMI, and therapy for diabetes or hyperlipidemia were not associated with PWV.

Multiple regression analyses were carried out by using the stepwise procedure in all diabetic patients (Table 2). The analysis included PWV as a dependent variable and candidate risk factors (age, diabetes duration, systolic blood pressure, diastolic blood pressure, sTNF-R1, sTNF-R2, therapy for hypertension) as independent variables (Table 2). PWV was independently predicted by age ($F = 15.1$), systolic blood pressure ($F = 31.6$), and serum concentration of sTNF-R2 ($F = 5.2$), which explained 49.2% of the variability of PWV in our diabetic patients. Other variables including diabetes duration, diastolic blood pressure, therapy for hypertension, and sTNF-R1 were not independently associated with PWV in our nonobese Japanese type 2 diabetic patients. Finally, smoking status and BMI were incorporated as candidate risk factors. PWV was independently predicted by age ($F = 15.1$), systolic blood pressure ($F = 31.6$), and serum concentration of sTNF-R2 ($F = 5.2$), which explained 49.2% of the variability of PWV in the patients. Smoking status ($F = 3.1$) and BMI ($F = 3.2$) were not independently associated with PWV in our patients.

4. Discussion

The main novel finding in the present study is that sTNF-R2 is strongly and independently associated with brachial-ankle PWV in nonobese Japanese type 2 diabetic patients.

Type 2 diabetes mellitus is a heterogeneous syndrome characterized by insulin resistance and/or defective insulin secretion [22]. As distinct from white populations, Japanese type 2 diabetic patients are unique in that they are not always obese, but some individuals are both insulin sensitive and insulin resistant [23-25]. The present study reconfirmed that 36% of the nonobese Japanese type 2 diabetic patients were insulin resistant.

Atherosclerosis is the leading cause of mortality and morbidity in subjects with type 2 diabetes mellitus. Although the mechanisms by which atherosclerosis occurs are not fully clarified, it has been shown that most clinical events result from mild to moderate arterial lesions that abruptly progress to severe obstructions [26]. Thus, detecting the early stage of atherosclerosis is considered to be the first line to clarify the mechanisms responsible for the evolution of atherosclerosis in type 2 diabetic patients.

Pulse wave velocity, a measure of aortic distensibility, is a noninvasive method to detect the early stage of atherosclerosis in humans. There are some reports suggesting that gender per se might affect the value of PWV [27-29]. We could not, however, find any significant relationship between PWV and gender in our patients.

Pulse wave velocity is shown to predict mortality in patients with hypertension and older healthy individuals, independently of known confounding factors [30]. Several studies have demonstrated that PWV correlates with diabetic complications. Tanokuchi et al [31] reported that PWV is related to serum creatinine level in patients with type 2 diabetes mellitus. Okada et al [32] showed the relationship between PWV and autonomic neuropathy in type 2 diabetic patients. Aso et al [33] demonstrated that PWV was associated with retinopathy and albuminuria in type 2 diabetic patients. PWV is also confirmed to be highly predictive of cardiovascular mortality in subjects with type 2 diabetes mellitus [5]. Thus, it may be hypothesized that micro- and macrovascular complications of type 2 diabetes mellitus share the common pathophysiologic mechanisms. This hypothesis is supported by the report from Neil et al [34] that microalbuminuria, a component of microvascular disease, has strongly and independently been associated with the development of cardiovascular disease and mortality in type 2 diabetic patients. Retinopathy, another microvascular complication, has been shown to be associated with increased cardiovascular and all-cause mortality risk in type 2 diabetic patients [35].

It is well recognized that low-grade inflammation per se seems to have a major role in the pathogenesis of

Table 3
Correlation of brachial-ankle PWV with measures for variables in 52 diabetic patients who have not received antihypertensive medications

	Univariate		Multivariate
	r	*P*	F
Age	0.291	.038	2.8
Diabetes duration	0.086	.537	–
Systolic blood pressure	0.533	<.001	15.2
Diastolic blood pressure	0.297	.034	1.6
TNF-α	0.198	.157	–
sTNF-R1	0.465	<.001	0.5
sTNF-R2	0.482	<.001	11.2
Gender	0.021	.879	–
Smoking	0.084	.549	–
BMI	−0.045	.747	–
Fasting glucose	−0.026	.853	–
HbA_{1c}	0.004	.974	–
Insulin	0.035	.804	–
HOMA-IR	−0.003	.982	–
Triglycerides	−0.018	.896	–
Total cholesterol	−0.144	.303	–
HDL cholesterol	−0.074	.599	–
LDL cholesterol	−0.153	.275	–
Serum creatinine	0.180	.198	–
Therapy for diabetes	−0.031	.827	–
Therapy for triglyceride	0.074	.598	–
Therapy for cholesterol	−0.021	.884	–

atherosclerosis and diabetes [36]. Ridker et al [37] showed that increased levels of inflammatory markers such as the high-sensitivity C-reactive protein (CRP) and interleukin 6 (IL-6) can predict increased risk of cardiovascular disease in humans. Serum IL-6 is shown to be predictive of the development of type 2 diabetes mellitus in women [38]. Stehouwer et al [39] confirmed that increased urinary albumin excretion, endothelial dysfunction, and chronic inflammation are interrelated processes that are associated with risk of death in type 2 diabetic patients. In the present study, however, we could not find any significant relationships between PWV and serum concentrations of CRP or IL-6 in our diabetic patients (data not shown). It may be argued that antihypertensive medications affect PWV by altering blood pressure in our patients. We therefore investigated 52 patients who have not received antihypertensive medications and found that PWV was independently predicted by the level of systolic blood pressure ($F = 15.2$) and sTNF-R2 ($F = 11.2$), which explained 35.6% of the variability of PWV (Table 3). This finding also supports our idea that TNF system activity per se plays an important role in PWV in Japanese type 2 diabetic patients.

Tumor necrosis factor α, the proinflammatory cytokine, seems to be associated with the progression of atherosclerosis in type 2 diabetes mellitus. There is, however, a paucity of the literature regarding the relationship between TNF-α and atherosclerosis in type 2 diabetic patients. As an index of TNF-α system activities, we measured serum TNF-α, serum sTNF-R1, and serum sTNF-R2 and found that serum sTNF-R2 is strongly and independently associated with PWV in nonobese Japanese type 2 diabetic patients. However, we could not find any independent relationship between PWV and serum TNF-α. It should be noted that TNF receptor levels remain elevated for a longer time than TNF-α itself and TNF receptors might reflect the degree of TNF-α activation more accurately than the measurement of TNF-α itself. Soluble TNF receptor is thus suggested to be a more valuable factor for monitoring the degree of TNF-α system activity in humans.

The mechanisms by which TNF-α system activities are associated with PWV in nonobese Japanese type 2 diabetic patients are not known at present. There is some evidence that TNF-α is associated with the evolution of atherosclerosis. TNF-α has been shown to contribute to the synthesis of inflammatory markers such as CRP and fibrinogen in liver [40], to mediate chemotaxis of monocytes and fibroblasts [41], and to enhance the expression of vascular cell adhesion molecules such as intercellular adhesion molecule 1 [42]. Irrespective of this, our present study showed that sTNF-R2 but not sTNF-R1 was independently associated with PWV. The validity of the present study is supported by the recent longitudinal investigation by Shai et al [9], who showed that sTNF-R2 is an independent predictor of CHD events in patients with type 2 diabetes mellitus.

The reason why TNF-R2 but not TNF-R1 was associated with PWV in our patients remains to be clarified. These 2 receptors seem to differ in signaling and functional properties [43]. Most biological responses such as cytotoxicity and nuclear factor κB activation are mediated by TNF-R1 but not by TNF-R2 [44]. There are some data available regarding the potential role of TNF-R2 in studies in humans. TNF-α has shown to up-regulate TNF-R2 expression in humans [45]. Obese subjects are shown to overexpress TNF-α and TNF-R2 in adipose tissue and have higher levels of TNF-R2 compared with lean subjects [46,47]. In contrast, we previously demonstrated that plasma TNF-R2 but not TNF-R1 was significantly higher in patients with bulimia nervosa [18]. Thus, it might be suggested that the adipose tissue is not the immediate source of TNF-α in our nonobese Japanese patients with type 2 diabetes mellitus. Recent studies have demonstrated that the binding of advanced glycation end products to specific cell-surface receptor molecules expressed on kidney cells can induce local cytokine and initiate local inflammatory reaction [48]. Angiotensin II, a substance that is associated with the development of renal injury in diabetic patients is shown to up-regulate expression of TNF-α [49]. The source of TNF-α in our patients, however, has yet to be determined. It should be noted that macrophages from diabetic patients release more TNF-α than do control macrophages [50]. Furthermore, high glucose can activate monocytes and induce the expression of TNF-α via oxidant stress and nuclear factor κB transcription factor [51].

In summary, although our present study was performed on a limited number of patients without major clinical signs of macrovascular complications, serum sTNF-R2 is likely to be involved in the brachial-ankle PWV in nonobese Japanese type 2 diabetic patients.

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