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ORIGINAL ARTICLE

Association between interleukin-19 and angiopoietin-2 with vascular complications in type 2 diabetes

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ABSTRACT

Aims/Introduction: The aim of the present study was to investigate the role of interleukin-19 (IL-19) in angiogenesis of type 2 diabetes mellitus with complications, and to assess the relationship of serum IL-19 and angiopoietin-2 (Ang-2) in type 2 diabetes mellitus.

Materials and Methods: The group studied comprised of 240 patients with type 2 diabetes mellitus (132 men and 108 women), and included macrovascular complications, microvascular complications and type 2 diabetes mellitus without vascular complications. The control group consisted of 50 healthy blood donors. All participants were evaluated for: IL-19, Ang-2, fasting plasma glucose, fasting insulin and glycosylated hemoglobin.

Results: The serum IL-19 levels of type 2 diabetes mellitus patients with angiopathy were found to be significantly higher compared with patients without angiopathy. IL-19 levels were significantly positively correlated with Ang-2, homeostasis model assessment for insulin resistance and glycosylated hemoglobin ($r = 0.769$, 0.523 and 0.491, respectively, $P < 0.01$). In multivariable logistic regression analysis, IL-19 levels ($P = 0.01$) were found to be independently associated with patients with type 2 diabetes mellitus complications.

Conclusions: These data are the first to implicate the association between the IL-19 and type 2 diabetes mellitus with vascular complications. IL-19 is significantly positively correlated with Ang-2. The potential role of IL-19 and Ang-2 in the pathogenesis of vascular complications in type 2 diabetes could warrant further study.

INTRODUCTION

Diabetes mellitus has become a global health problem, and its prevalence is expected to increase to 366 million worldwide by 2030[1]. Diabetes mellitus, especially type 2 diabetes mellitus, is a metabolic disease characterized by chronic hyperglycemia, which mainly results from a deficiency in peripheral insulin effects (insulin resistance). However, morbidity and mortality from diabetes are mainly attributed to the development of both macrovascular and microvascular complications that rapidly leads to premature death[2,3]. Vascular complications dominate the lifespan and the quality of life in type 2 diabetes mellitus patients, and it is important to prevent the development and progression of these complications[4]. Chronic inflammation, characterized by elevated circulating levels of inflammatory

markers, appears to play a critical role in the pathogenesis of type 2 diabetes mellitus and its associated complications[5,6]. Recent accumulating evidence shows that chemokines, adhesion molecules, growth factors, angiopoietins and interleukins are involved in the participation of immune-mediated inflammatory processes in the pathophysiology of diabetes mellitus and its complications[7,8].

The angiopoietins are a family of seven secreted glycoprotein ligands, angiopoietin-1 to -7, originally identified as important in blood vessel formation. The best characterizations of these ligands are angiopoietin-1 (Ang-1) and angiopoitein-2 (Ang-2). Ang-2 is expressed by endothelial cells and acts as an antagonist for receptor Tie-2. Ang-2 has been reported to completely disrupt protective Tie-2 signaling in numerous studies[9–11].

Interleukin-19 (IL-19) is a newly discovered cytokine belonging to the IL-10 family. This protein can induce the production

of IL-10 from human peripheral blood mononuclear cells[12,13]. IL-19 is a potent inducer of the T-helper2 response, and has been implicated in a wide variety of allergies (i.e. asthma and atopic dermatitis)[14–16], type 1 diabetes[17] and cardiovascular disease[18,19].

Our previous study showed that Ang-2 is closely related to vascular complications of type 2 diabetes mellitus[20]. However, how IL-19 functions in type 2 diabetes mellitus, and whether Ang-2 and IL-19 play an important role in the pathogenesis of type 2 diabetes mellitus have been scarcely reported. The purpose of the present study was to extend those initial observations, and to determine the concentrations of IL-19 in type 2 diabetes mellitus and its complications, and to investigate the relationship of serum Ang-2 and IL-19 in type 2 diabetes mellitus.

MATERIAL AND METHODS

Patient selection
A total of 240 patients with type 2 diabetes mellitus (132 men and 108 women) treated at Binhai County Hospital, Jiangsu Province, China, were recruited in the present study. The average patient age was 62 ± 11 years. The duration of diabetes development from 6 to 14 years. The patients were diagnosed according to World Health Organization criteria[21]. Primary diagnoses (by clinical, electrocardiogram and imaging diagnosis) included macrovascular complications ($n = 76$, including heart disease, cerebrovascular disease and peripheral arterial disease), microvascular complications ($n = 110$, including diabetic nephropathy [urinary albumin >30 mg/24 h], as well as diabetic retinopathy and diabetic peripheral neuropathy) and diabetic without vascular complications ($n = 54$). According to the number of macrovascular and microvascular injuries, respectively, patients with macrovascular or microvascular complications were divided into the following groups respectively: one kind of macrovascular complications group ($n = 25$) or microvascular complications group ($n = 50$), two kinds of macrovascular complications group ($n = 30$) or microvascular complications group ($n = 35$), and three kinds of macrovascular complications group ($n = 21$) or microvascular complications group ($n = 25$). The control group consisted of 30 men and 20 women, and the average age was 60 ± 15 years. The exclusion criteria included patients with evidence of neoplastic disease, acute or chronic infectious disease, and significant hepatic and renal disease. The study was approved by the Human Investigation Committee of BinHai County Hospital, and informed consent for all participants was obtained.

Laboratory analysis
Venous blood samples were obtained from all participants on hospital admission. All samples were collected in vacuum blood collection tubes with a clot activator, and immediately centrifuged at 1,500 *g* and 4°C for 10 min. Plasma was aliquoted and stored at −80°C until analysis.

Serum IL-19 and Ang-2 levels were determined by enzyme-linked immunosorbent assay using commercial kits and reagents (R&D Systems, Minneapolis, MN, USA). Glycosylated hemoglobin (HbA1c) was measured by liquid chromatography (G8-90SL;

Tosoh, Japan). Fasting plasma glucose and fasting insulin were measured by routine techniques. The homeostasis model assessment of insulin resistance index (HOMA-IR) was calculated by the following formula: HOMA-IR = (fasting plasma glucose × fasting insulin)/22.5.

Statistical analysis
The values are expressed as mean ± standard error of the mean. Comparisons of means between two groups were carried out using Student's *t*-test on test of normality and equality of variances. Correlations within each group were sought using Spearman's or Pearson's method. Multivariable logistic regression analyses were carried out to determine whether IL-19 levels were independently associated with vascular complications of type 2 diabetes. $P < 0.05$ was considered statistical significance. All analyses were carried out using SPSS 15.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Clinical and laboratory measures
A total of 240 patients with type 2 diabetes and 50 normal healthy controls were recruited. These participants were age and sex ratio comparable. As expected, HbA1c, fasting insulin, HOMA-IR, total cholesterol and triglyceride levels were higher, and high-density lipoprotein cholesterol was lower in the diabetic patients. Serum IL-19 levels was significantly higher in patients with type 2 diabetes mellitus than in healthy controls, and there were differences in the diabetic patient subgroups (Table 1).

Relationship of IL-19 and the number of microvascular or macrovascular Injuries in type 2 diabetes mellitus
In the type 2 diabetes mellitus cohort, 186 patients had suffered vascular complications. In type 2 diabetes mellitus patients, along with the increased numbers of microvascular or macrovascular complications, IL-19 concentration also increased. IL-19 levels were also significantly different ($P < 0.05$; Table 2,3).

Correlations
Serum IL-19 levels positively correlated with HOMA-IR and HbA1c ($r = 0.523$ and 0.491, respectively, $P < 0.01$; Figure 1).

Association between serum IL-19 and vascular complications
In multivariable logistic regression analysis, IL-19 levels (95% confidence interval 22.4–61.4; $P = 0.01$) were independently associated with type 2 diabetes mellitus patients with vascular complications. IL-19 was positively correlated with microangiopathy and macroangiopathy numbers ($r = 0.503$ and 0.618, respectively; $P < 0.05$) after adjustment for age, sex, hypertension and blood fat.

Ang-2 was associated with IL-19 levels in type 2 diabetes mellitus
Previous studies[20] observed that Ang-2 concentrations were elevated in patients with type 2 diabetes. In the present study,

Table 1 | Clinical characteristics and research indexes in the study groups

	Controls	T2DM	T2DM with macrovascular complications	T2DM with microvascular complications	T2DM without vascular complications	P-value
n	50	240	76	110	54	—
Age (years)	60 ± 15	62 ± 11	64 ± 5	69 ± 4	66 ± 5	0.125
Sex(males/females)	30/20	132/108	35/31	58/52	39/25	0.314
Duration of diabetes (years)	—	9 (6–14)	10 (6–12)	10 (7–14)	8 (7–13)	0.210
BMI (kg/m^2)	25 ± 5	28 ± 6	29 ± 5	26 ± 4	25 ± 6	0.001
SBP (mmHg)	128 ± 16	139 ± 15	137 ± 16	138 ± 15	136 ± 15	0.060
DBP (mmHg)	77 ± 7	78 ± 11	75 ± 9	76 ± 8	75 ± 7	0.213
TC (mmol/L)	4.8 ± 1.0	5.7 ± 1.6	5.5 ± 1.5	5.9 ± 1.1	5.6 ± 1.2	0.911
Triglycerides (mmol/L)	1.5 ± 0.5	2.8 ± 1.6	2.0 ± 1.2	2.5 ± 1.6	2.4 ± 1.5	0.895
HDL cholesterol (mmol/L)	1.6 ± 0.5	1.2 ± 0.4	1.3 ± 0.2	1.1 ± 0.5	1.3 ± 0.3	<0.001
LDL cholesterol (mmol/L)	3.2 ± 0.8	2.9 ± 0.8	2.5 ± 0.9	2.3 ± 0.7	2.6 ± 1.0	0.058
Cystatin C (mg/L)	0.6 ± 0.04	1.08 ± 0.07	1.32 ± 0.09	1.05 ± 0.07	0.87 ± 0.05	0.03
IL-19 (pg/mL)	16.2 ± 8.5	41.9 ± 11.9	53.7 ± 14.3	40.5 ± 12.3	30.4 ± 9.2	<0.001
Ang-2 (ng/mL)	0.8 ± 0.2	2.2 ± 0.7	2.9 ± 0.7	2.2 ± 0.6	1.6 ± 0.4	0.001
FBG (mmol/L)	5.1 ± 0.5	9.4 ± 2.3	10.5 ± 2.8	9.2 ± 2.3	8.5 ± 1.7	<0.001
FINS (mU/L)	8.0 ± 1.3	18.1 ± 2.7	18.7 ± 2.4	15.5 ± 2.1	20.1 ± 3.8	<0.001
HOMA-IR	1.8 ± 0.1	7.6 ± 0.2	8.7 ± 0.3	6.3 ± 0.2	7.6 ± 0.3	<0.001
HbA1c (%)	4.8 ± 0.9	9.5 ± 2.1	11.3 ± 3.5	9.7 ± 2.7	8.9 ± 2.1	<0.001

Data are presented as mean ± standard deviation. Ang-2, angiopoietin-2; BMI, body mass index; DBP, diastolic blood pressure; FINS, fasting insulin; FPG, fasting plasma glucose; TC, total cholesterol; HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment for insulin resistance; IL-19, interleukin-19; LDL, low-density lipoprotein; SBP, systolic blood pressure; T2DM, type 2 diabetes mellitus.

we also observed a positive correlation between Ang-2 and IL-19 levels in type 2 diabetes mellitus patients ($r = 0.769$; $P < 0.01$).

DISCUSSION

Diabetes is associated with a range of vascular diseases, including atherosclerosis, myocardial infarction and heart failure resulting from endothelial dysfunction[22]. Ang-2 is a glycoprotein exclusively expressed by endothelial cells that acts as an antagonist for Tie-2. Ang-2 can inhibit Tie2 phosphorylation, damage blood vessels, promote blood vessel structure relaxation, and relieve inhibition effects on perivascular cells and extracellular matrix to the endothelium, causing vascular endothelial injury and pathological angiogenesis[11,23]. In previous studies[20], we have shown that serum Ang-2 levels were positively associated with microvascular and macrovascular lesions in patients with type 2 diabetes mellitus.

IL-19 was discovered as a member of the IL-10 family, and it shares 20% amino acid identity with IL-10, but does not engage the IL-10 receptor. IL-19 can be detected in human monocytes, and B and T lymphocytes, and IL-19 expression can be upregulated in these cells by stimulation[24,25]. IL-19 expression is reported to be restricted to immune cells, and all of our knowledge regarding the function of this cytokine comes from experiments carried out in inflammatory cells.

Patients with diabetes are at risk for microvascular and macrovascular lesions[26]. Inflammatory events are thought to

Table 2 | Results of the different numbers of microvascular injuries in type 2 diabetes mellitus

| n	No. microvascular complications	IL-19 (pg/mL)
50	1	33.1 ± 9.3
35	2	40.5 ± 12.7
25	3	48.1 ± 14.9

IL-19, interleukin-19.

Table 3 | Results of the different numbers of macrovascular injuries in type 2 diabetes mellitus

n	No. macrovascular complications	IL-19 (pg/mL)
25	1	41.7 ± 10.3
30	2	52.5 ± 15.7
21	3	66.9 ± 16.9

IL-19, interleukin-19.

occur in all diabetic complications[27]. IL-19 is considered by some investigators to have an anti-inflammatory effect[28,29]. However, IL-19 is also capable of activating monocytes to release IL-6, tumor necrosis factor TNF-α, IL-8 and reactive oxygen species, and has been implicated in the pathogenesis of sepsis-induced organ injury[30,31]. Cuneo et al.[18] also reported that IL-19 can be
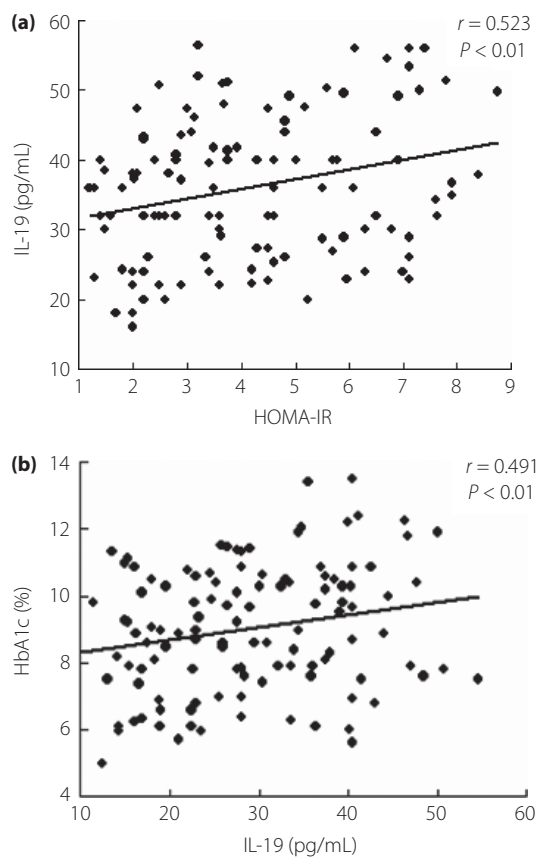
Figure 1 | Serum (a) interleukin-19 (IL-19) levels and (b) Ang-2 were positively correlated with homeostasis model assessment for insulin resistance (HOMA-IR) and glycosylated hemoglobin (HbA1c), respectively.

induced by inflammatory cytokines and inflammatory stimuli, and IL-19 is expressed in injured and stimulated vascular smooth muscle cells. The present study found that IL-19 was elevated in patients with diabetes, and it was positively associated with the number of concurrent microvascular and macrovascular lesions in patients with type 2 diabetes mellitus. The present results showed that IL-19 play a critical role in the pathogenesis of vascular disease in diabetes mellitus.

Ang-2 is stored in endothelial cell Weibel–Palade bodies, and high concentrations of Ang-2 produce marked impairment of endothelial function. Ang-2 is known to increase levels of pro-inflammatory cytokines[32]. In the present study, serum IL-19 levels were positively correlated with Ang-2, and also a positive correlation between IL-19 and HbA1c, HOMA-IR was observed, which shows that IL-19 was closely associated with glucose metabolism disorders and vascular lesions in patients with type 2 diabetes mellitus. The present results suggest that long-term hyperglycemia increases IL-19 expression through stimulating endothelial cells. Furthermore, insulin resistance and insufficient insulin signaling in patients with type 2 diabetes mellitus might also lead to the increased expression of IL-

19. Additionally, the increased expression of Ang-2 in response to high glucose might result in increased expression of IL-19. These data provide evidence to suggest that high glucose-induced IL-19 production might be mediated by the induction of Ang-2 in vascular endothelial cells. However, the mechanism requires further investigation.

The high concentrations of glycation end-products in diabetic patients can upregulate inflammatory cytokines expression by stimulating endothelial cells. Conversely, inflammatory cytokines can stimulate endothelial cell proliferation and induce local inflammation, accelerating the generation and development of vascular disease[33,34]. In the present study, we found that IL-19 levels gradually increased in the following order: type 2 diabetes mellitus patients without vascular complications group, the microangiopathy group and the macroangiopathy group. Importantly, IL-19 was significantly higher among diabetic patients with macrovascular complications when compared with those without macrovascular complications. In the multivariable logistic regression analysis, IL-19 levels were independently associated with type 2 diabetes mellitus patients with vascular complications. This showed that the extent of vascular damage might be closely linked to IL-19 levels. Several hypotheses can be made on the role of IL-19 levels in diabetic vasculopathy. First, IL-19 alone might exert pro-angiogenic activity. Second, high IL-19 levels might result in local inflammation. Finally, IL-19 expression contributes to Ang-2-induced impairment of endothelial responses. IL-19 and Ang-2 could emerge with a direct role in the acceleration of vascular injury, in addition to the pro-inflammatory effect on this pathophysiology.

It has to be stressed that our current study was limited. The sample size of the present cross-sectional study was relatively small. In addition, the correlation between IL-19 and Ang-2 in the present clinical study provides correlated evidence, but does not address the cause–effect relationship in type 2 diabetes.

In summary, the present data show that levels of serum IL-19 and Ang-2 were significantly elevated in patients with type 2 diabetes mellitus. Specifically, they were positively correlated with the numbers of vascular complications of type 2 diabetes mellitus. Therefore, these observations provide Ang-2 and IL-19 as further insights into the possible effectiveness of anti-inflammatory therapy in the treatment and prevention of diabetic angiopathy.

DISCLOSURE

The authors declare no conflict of interest.

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