Journal of Diabetes and Its Complications 29 (2015) 568–571

Contents lists available at ScienceDirect

Journal of Diabetes and Its Complications

journal homepage: WWW.JDCJOURNAL.COM

Serum angiopoietin-2 is associated with angiopathy in type 2 diabetes mellitus

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ARTICLE INFO

Article history:
Received 6 November 2014
Received in revised form 9 February 2015
Accepted 13 February 2015
Available online 20 February 2015

Keywords:
Angiopoietin-1
Angiopoietin-2
Glycosylated hemoglobin
Diabetes mellitus
Vascular complications

ABSTRACT

Purpose: The aim of the present study was to investigate the association of serum levels of angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2) with angiopathy in type 2 diabetes mellitus (T2DM).
Methods: The group studied comprised of 120 patients with T2DM (68 males and 52 females), included macroangiopathy complications, microvascular disease and diabetic without vascular disease. The control group consisted of 50 healthy blood donors. Ang-1, Ang-2, fasting plasma glucose (FBG), fasting insulin (FINS) and HbA1c were assessed.
Results: The serum Ang-2 levels of T2DM patients with angiopathy were found to be significantly higher compared to patients without angiopathy. Ang-2 levels were significantly positively correlated with homeostasis model assessment for insulin resistance (HOMA-IR) and glycosylated hemoglobin A1c (HbA1c) ($r = 0.577$ and 0.504, respectively, $P < 0.01$). In contrast, there was no significant correlation between Ang-1 levels and HOMA-IR ($P > 0.05$). In multivariable logistic regression analysis, Ang-2 levels ($P = 0.02$) were found to be independently associated with patients with T2DM angiopathy.
Conclusions: An association between the Ang-2 and T2DM with vascular complications was found.

1. Introduction

Type 2 diabetes mellitus (T2DM) 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 (Jaumdally, Goon, Varma, et al., 2010; Rasul, Reiter, Ilhan, et al., 2011). Insulin-producing beta-cells and endothelial cells in the pancreatic islets of Langerhans exchange bidirectional signals necessary for development, differentiation, and the proper function of both endocrine and vascular compartments (Rasul et al., 2011). Beta-cells secreted angiogenic factors, like vascular endothelial growth factor, as well as anti-angiogenic factors, like angiopoietin (Calderari et al., 2012).

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) (Davis, Aldrich, Jones, et al., 1996; Valenzuela, Griffiths, Rojas, et al., 1999). Ang-1, a secreted 70 kDa glycoprotein constitutively expressed by pericytes and vascular smooth muscle cells, is a major agonist for the tyrosine kinase receptor Tie-2. Binding of Ang-1 to Tie-2 promotes vessel integrity, inhibits vascular leakage, and suppresses inflammatory gene expression. On the other hand, Ang-2 is also expressed by endothelial cells and acts as an antagonist for Tie-2. Ang-2 has been reported to completely disrupt protective Tie-2 signaling in numerous studies (Chen, Guo, & Chen, 2013; Thomas & Augustin, 2009).

Recent results revealed that Ang-2 is closely related to abnormal vascular endothelial inflammation, insulin resistance and vascular injury. However, whether Ang-1 and Ang-2 levels are relevant to T2DM patients with vascular complications remains unknown. Therefore, the aim of this study was to investigate the association of serum Ang-1 and Ang-2 with T2DM patients with vascular lesions and insulin resistance.

2. Material and methods

2.1. Patient selection

A total of 120 patients with T2DM (68 males and 52 females) treated at BinHai County People's hospital were recruited in this study. The average patient age was 62 ± 11 years old. The patients were diagnosed according to WHO criteria (Geneva, 1999). Primary diagnoses (by clinical, ECG, and imaging diagnosis) included macroangiopathy complications ($n = 32$, including heart disease, cerebrovascular disease, and peripheral arterial disease), microvascular disease ($n = 52$, including diabetic nephropathy (urinary albumin > 30 mg/24 h)), as

Conflict of interest: None.

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http://dx.doi.org/10.1016/j.jdiacomp.2015.02.006
1056-8727/

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

	Controls	T2DM	T2DM With macroangiopathy complications	T2DM with microangiopathy complications	T2DM without angiopathy complications	P-value
n	50	120	32	52	36	–
Age (years)	60 ± 6	62 ± 11	64 ± 5	69 ± 4	66 ± 5	0.125
Sex (males/females)	30/20	68/52	17/15	28/24	23/13	0.314
BMI (kg/m2)	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
Ang-1 (ng/ml)	19.5 ± 1.7	19.6 ± 1.8	19.1 ± 1.7	20.2 ± 2.0	19.8 ± 2.1	0.420
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 ± SD, BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Ang, angiopoietin; HOMA-IR, homeostasis model assessment for insulin resistance.
* Significant difference between patients with T2DM and controls but not between patient groups.

well as diabetic retinopathy and diabetic peripheral neuropathy) and diabetic without vascular disease (n = 36). According to the number of macrovascular and microvascular injuries respectively, patients with macrovascular or microvascular disease were divided into the following groups respectively: one kind of macrovascular disease group (n = 10) or microvascular disease group (n = 22), two kinds of macrovascular disease group (n = 15) or microvascular disease group (n = 18), and three kinds of macrovascular disease group (n = 7) or microangiopathy group (n = 12). The control group consisted of 30 males and 20 females, and the average age was 60 ± 15 years old. The exclusion criteria included patients with evidence of neoplastic disease, chronic inflammation (except vascular injuries), significant hepatic and renal disease. The study was approved through the local research ethics committee, and informed consent for all subjects was obtained.

2.2. Laboratory analysis

Venous blood samples were obtained from all subjects upon hospital admission. All samples were collected in vacuum blood collection tubes with a clot activator and immediately centrifuged at 1000 × *g* and 4 °C for 20 min. Plasma was aliquoted and stored at −70 °C until analysis.

Serum Ang-1 and Ang-2 levels were measured by the enzyme-linked immunosorbent assay (ELISA) using commercial kits and reagents (R&D Systems, Minneapolis, MN, USA). HbA1c was measured by liquid chromatography (G8-90SL, Tosoh, Japan). Fasting plasma glucose (FBG) and fasting insulin (FINS) were measured by routine techniques. The homeostasis model assessment of insulin resistance index (HOMA-IR) was calculated by the following formula: HOMA-IR = (FBG × FINS)/22.5.

2.3. Statistical analysis

The values are expressed as mean ± S.D. Comparisons of means between two groups were performed using Student's t-test upon 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 performed to determine whether Ang-1 and Ang-2 levels were independently associated with T2DM with vascular complications during hospitalization. $P < 0.05$ was considered a statistical significance. All analyses were performed using SPSS 15.0 (SPSS, Inc., Chicago, IL, USA).

3. Results

3.1. Clinical and laboratory measures

A total of 120 patients with type-2 diabetes and 50 normal healthy controls were recruited. These subjects were age and sex ratio comparable. As expected, HbA1c, FINS, HOMA-IR, TC, and triglyceride levels were higher, and HDL cholesterol was lower in the diabetic patients. Serum Ang-2 levels, but not Ang-1 levels, were significantly higher in patients with T2DM than in healthy controls, and there were differences between the diabetic patient subgroups (Table 1). There were no significant relationships between Ang-1 and Ang-2 levels with age, sex, and body mass index.

3.2. Relationship of Ang-2 and the number of microvascular or macrovascular injuries in T2DM

In our T2DM cohort, 84 patients had suffered vascular complications. In T2DM patients with increased microvascular or macrovascular disease, Ang-2 concentration also increased. Ang-2 levels were also significantly different ($P < 0.05$). The concentration of concurrent Ang-1 levels was not significantly different related to different microangiopathy or macroangiopathy numbers ($P > 0.05$) (Tables 2, 3).

3.3. Correlations

Serum Ang-2 levels positively correlated with HbA1c and HOMA-IR ($r = 0.504$ and 0.577 respectively, $P < 0.01$; Fig. 1). However, serum Ang-1 levels did not significantly correlate with HbA1c and HOMA-IR ($P > 0.05$). BMI, as well as systolic and diastolic blood pressure did not significantly correlate with Ang-2 levels.

Table 2
Results of the different numbers of microvascular injuries in T2DM.

Numbers of microvascular	Ang-1 (μg/L)	Ang-2 (μg/L)
1	20.2 ± 1.9	2.1 ± 0.3
2	20.3 ± 2.0	2.5 ± 0.7
3	20.1 ± 2.1	2.9 ± 0.9

Table 3
Results of the different numbers of macrovascular injuries in T2DM.

Numbers of macrovascular	Ang-1 (μg/L)	Ang-2 (μg/L)
1	19.0 ± 1.4	2.5 ± 0.5
2	19.2. ± 1.8	2.9 ± 0.8
3	19.1 ± 1.9	3.4 ± 0.7