

Plasma Ephrin-A1 level in a cohort of diabetic retinopathy patients

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Abstract

Background: To determine plasma Ephrin-A1 and VEGF 165 levels in a cohort of diabetic retinopathy patients.

Methods: Plasma ephrin-A1 and VEGF 165 levels in fifty-five subjects including 19 individuals without diabetes (non-DM), 16 patients with diabetes (DM) but without diabetic retinopathy, and 20 patients with diabetic retinopathy (DR), were determined by ELISA. Serum creatinine, total cholesterol, fasting blood glucose and HbA1c were also measured. Mann-Whitney U analysis, independent sample T analysis and Spearman correlation coefficient analysis were used for data analysis.

Results: Ephrin-A1 expression could be detected in human plasma with an average of 1.52 ± 0.43 (mean \pm SEM) ng/ml. In DR subjects, the plasma ephrin-A1 concentration was 3.63 ± 4.63 ng/ml, which was significantly higher than that of the other two groups (non-DM: 0.27 ± 0.13 ng/ml, DM: 0.35 ± 0.34 ng/ml). The expression of VEGF 165 in human plasma was 34.00 ± 42.55 pg/ml, with no statistical difference among the three groups. There was no correlation between Ephrin-A1 and VEGF 165 in human plasma.

Conclusions: Plasma ephrin-A1 was highly expressed in patients with diabetic retinopathy, and there was no difference of plasma VEGF 165 expression in patients with diabetic retinopathy compared to the other two groups, suggesting that changes of plasma ephrin-A1 may be a more sensitive biomarker than plasma VEGF 165 in detecting diabetic retinopathy.

Background

It is estimated that there are 382 million diabetic patients worldwide, and about one third of these patients have diabetic retinopathy (DR), part of them have diabetic macular edema. DR causes visual impairment in about 37 million people around the world (1). About 60% of type 2 diabetes patients present DR of varying degrees 20 years after being diagnosed with diabetes. DR is induced by hyperglycemia, which leads to retinal vascular endothelial injury, retinal vascular loss, ischemia, and changes of leukocyte adhesion (2, 3). Consequently, production of precursor substances of various angiogenic factors and inflammatory cytokines increased which induced abnormal neovascularization (4) and microvascular dysfunction.

In the pathogenesis process of DR, endothelial-derived growth factor (VEGF) is a major cytokine that promotes neovascularization, which leads the destruction of blood retinal barrier and indirectly promoting the progress of DR. Several spliced isoforms of VEGF present in human body with 121, 145, 165, 183,189, and 206 amino acids in length (5). VEGF₁₆₅ appears to be the most abundant and potent isoform in inducing neovascularization, followed by VEGF₁₂₁ and VEGF₁₈₉ (5, 6). *In vivo* and *in vitro* experiments have shown that ischemia and hypoxia induces VEGF expression, which causes retinal neovascularization (7). In addition, it has been shown that VEGF levels in the vitreous of DR patients were higher than those in the control group (8). Intravitreal injection of anti-VEGF drugs has a certain

therapeutic effect on PDR patients (9, 10). However, there are disadvantages that anti-VEGF drugs require multiple intravitreal injections for the treatment of DR, that maybe causing preretinal tissue shrinkage and retinal detachment (11). In addition, anti-VEGF drugs have adverse effects such a mild cerebrovascular accident and myocardial infarction. Therefore, it is necessary to find more appropriate approaches or new therapeutic targets.

Ephrins and the Eph receptors have been identified as key regulators of angiogenesis (12). Class A Eph receptors have been shown to regulate postnatal angiogenesis in adults. Earlier studies have shown that ephrin-A1 stimulates the migration of cultured endothelial cells and induces corneal angiogenesis (13). Expression of ephrin-A1 was found in the process of embryonic vascular development (12) and tumor growth (14). These evidences indicate that ephrin-A1 plays an important role in angiogenesis. We became interested whether ephrin-A1 is expressed in human plasma, and whether the plasma ephrin-A1 expression was altered in diabetic retinopathy patients.

Methods

Subjects

This study was approved by institutional ethics committee in the Fenghua Hospital of Traditional Chinese Medicine. Informed consents in written format to publish these date were collected from each patient, who agreed and signed the consent to participate statement. The recruited patients were enrolled between August 1, 2018 and October 31, 2019 with the following inclusion criteria:

1. Over 40 years of age.
2. Clinical diagnosis of primary angle-closure suspect, diabetes mellitus and DR.
3. Patients agreed to provide informed consent.

The definition of primary angle-closure suspect was in accordance with the International Society of Geographical and Epidemiologic Ophthalmology criteria (15). Diabetes mellitus was diagnosed according to the international standards (16). Diagnosis of DR was made based on an international standard (17).

The subjects with any diseases that presented neovascularization such as kidney disease and cancer, or any of those subjects received anti-VEGF therapy or operations were excluded.

A total of fifty-five patients from Fenghua District were recruited. Nineteen patients with primary angle-closure but without diabetes were enrolled in the non-DM group. Sixteen patients with primary angle-closure and diabetes were enrolled in DM group. Twenty patients with DR were enrolled in DR group.

After routine physical and eye examinations, electrocardiogram, clinical laboratory tests including liver and kidney function tests, routine ophthalmic examinations, fundus photograph and fluorescein angiography were performed.

The averaged duration time of type 2 DM in the diabetic group was 46 ± 18.5 months. The averaged duration time of type 2 DM with DR was 118 ± 49 months.

Collection of Blood Samples

The blood samples were collected using ethylenediaminetetraacetic acid (EDTA) as an anticoagulant from the peripheral vein of each patient and transferred to Department of Clinical Laboratory, which is a section of Fenghua Hospital of Traditional Chinese Medicine. Samples were centrifuged immediately for 15 minutes at 1000 g. Then, plasma was collected and stored at -80°C before enzyme-linked immunosorbent assay (ELISA) measurement.

All individuals were informed of the purpose of the study and their informed consent was obtained. This study followed the tenets of the Declaration of Helsinki and was approved by the ethics committee of the institutional Fenghua Hospital of Traditional Chinese Medicine.

Measurement of plasma Ephrin-A1 and VEGF₁₆₅

Ephrin-A1 was measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (LSBio, Seattle, WA, USA) and VEGF₁₆₅ was measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit (R&D systems, Minneapolis, MN, USA), following the instructions. Results were obtained by a multifunction microplate reader (Molecular Devices Inc., Sunnyvale, CA, USA). In addition, 6 ml venous blood of subjects was taken for measurement of fasting blood glucose, HbA1c, serum creatinine, and total cholesterol levels.

Statistical analysis

Statistical analyses were performed using SPSS 18.0 (SPSS Inc. Chicago, IL, USA). The data were shown as mean \pm SD. After inspecting the distribution of the data, we assessed statistical significances with Mann-Whitney U test, Kruskal-Wallis test or independent sample T test. Correlations between any two of parameters including plasma ephrin-A1, VEGF₁₆₅, fasting blood glucose levels, HbA1c, serum creatinine and total cholesterol were performed. BMI and the lipid parameters were examined by Spearman's correlation analysis. $P < 0.05$ was considered statistically significant.

Results

- Clinical and laboratory data were shown in Table 1. There was no statistical difference in age, sex, VEGF₁₆₅, serum creatinine and total cholesterol among the three groups of subjects. No relationship between Ephrin-A1 and VEGF₁₆₅ in human plasma had been found. There were differences in BMI, fasting blood glucose and HbA1c among the three groups. Compared to the DM subjects and the non-DM subjects, DR patients had higher averaged BMI, fasting blood glucose and HbA1c. These parameters in DM subjects were significantly higher than those of the non-DM subjects ($p < 0.05$).
- Ephrin-A1 could be measured in human plasma, the averaged concentration of ephrin-A1 was 1.52 ± 0.43 (mean \pm SEM) ng/ml. The concentration of ephrin-A1 in the DR subjects was significantly higher

than that of the other two groups ($p < 0.05$) (Fig. 1). This difference of the ephrin-A1 concentration was not found between the non-DM subjects and the DM subjects.

Plasma ephrin-A1 was not correlated with age, BMI, serum VEGF₁₆₅, serum creatinine, total cholesterol, fasting blood glucose and HbA1c in the DR patients, the DM subjects, and the non-DM subjects or all subjects.

	non-DM (n = 19)	DM (n = 16)	DR (n = 20)	P Value 1	P Value 2	P Value 3
Age (years)	60.53 ± 4.04	63.31 ± 4.97	60.25 ± 6.72	0.077	0.877	0.126
male/female	11/8	8/8	11/9			
BMI (kg/m ²)	22.01 ± 2.25	24.48 ± 3.17	24.32 ± 3.66	0.011	0.023	0.892
FPG (mmol/l)	5.57 ± 0.62	8.91 ± 3.04	8.16 ± 2.57	∞0.001	∞0.001	0.427
HbA1c(%)	5.33 ± 0.32	8.65 ± 2.56	8.22 ± 1.84	∞0.001	∞0.001	0.558
VEGF165(pg/ml)	34.79 ± 38.65	24.43 ± 17.33	40.91 ± 58.01	0.756	0.879	0.765
Ephrin-A1(ng/ml)	0.27 ± 0.13	0.35 ± 0.34	3.63 ± 4.63	0.756	∞0.001	∞0.001
Serum creatinine(µg/ml)	54.32 ± 12.74	68.88 ± 34.04	70.10 ± 26.63	0.193	0.019	0.369
Total cholesterol (mmol/l)	5.19 ± 1.09	5.41 ± 1.45	5.00 ± 1.00	0.683	0.351	0.648

P Value 1 indicates *P* value of the non-DM group vs the DM group; *P Value 2* indicates *P* value of the DM group vs the DR group; *P Value 3* indicates *P* value of the non-DM group vs the DR group.

FPG, fasting plasma glucose. Values are means ± SD.

The concentration of ephrin-A1 of the DR subjects was significantly higher than that of the other two groups ($p < 0.05$). There was no statistical difference in VEGF₁₆₅ among the three groups.

Discussion

- Interaction between EphA receptor tyrosine kinases (RTKs) and ephrinA ligands is necessary for inducing of maximal neovascularization by VEGF (18). EphA2 RTK is activated by VEGF through

induction of ephrinA1 ligand (18). Thus, we speculated that ephrin-A1 may be involved in the pathogenesis of DR and the ephrin-A1 concentrations in DR patients may be changed. To the best of our knowledge, no such research has been conducted before. Meanwhile, there were no reports about the association between ephrin-A1 and primary angle-closure suspects, or VEGF and primary angle-closure suspects in the literature.

- In this study, ephrin-A1 concentrations in the plasma of non-diabetic, diabetic and DR patients were measured in a cohort of subjects. The averaged ephrin-A1 concentration in human plasma was 1.52 ± 0.43 (mean \pm SEM) ng/ml, indicating ephrin-A1 was expressed in human plasma. We found ephrin-A1 concentration in the DR group was higher than that in the non-diabetic and the diabetic groups, suggesting ephrin-A1 may be involved in the development of DR. However, the detail role of ephrin-A1 in the pathogenesis of DR remains further studies.
- We also measured the plasma concentrations of VEGF₁₆₅ in these patients. We did not find difference in plasma VEGF₁₆₅ among the three groups. This result was inconsistent with a previous report that the plasma VEGF₁₆₅ concentration elevated in DR patients compared to non-DR controls (19). Further studies are needed to confirm the changes of VEGF₁₆₅ in the systemic circulation in DR patients.

Conclusions

This study is the first report that ephrin-A1 is presented in circulation. Furthermore, ephrin-A1 expression is elevated in DR patients, suggesting it may be involved in the pathogenesis of DR. Whether it may become a biomarker for the disease deserves further studies.

Abbreviations

VEGF endothelial-derived growth factor

DM diabetes

DR diabetic retinopathy

ELISA enzyme-linked immunosorbent assay

EDTA ethylenediaminetetraacetic acid

Declarations

Ethics approval and consent to participate

Ethics approval: This study was approved by institutional ethics committee in the Fenghua Hospital of Traditional Chinese Medicine. This study adhered to the tenets of the Declaration of Helsinki. Informed

consents in written format to publish these data were collected from each patient, who agreed and signed the consent to participate statement.

Consent for publication

Not applicable

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors report no conflicts of interest.

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Authors' contributions

YJ and BL conceived and designed the study. YH, QB and KWW acquired the data. YDZ and YKY performed the study. DNM analyzed the data and wrote the manuscript. BL revised the manuscript critically. YJ and BL contributed to the manuscript as the corresponding author. All authors read and approved the final manuscript.

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Figures

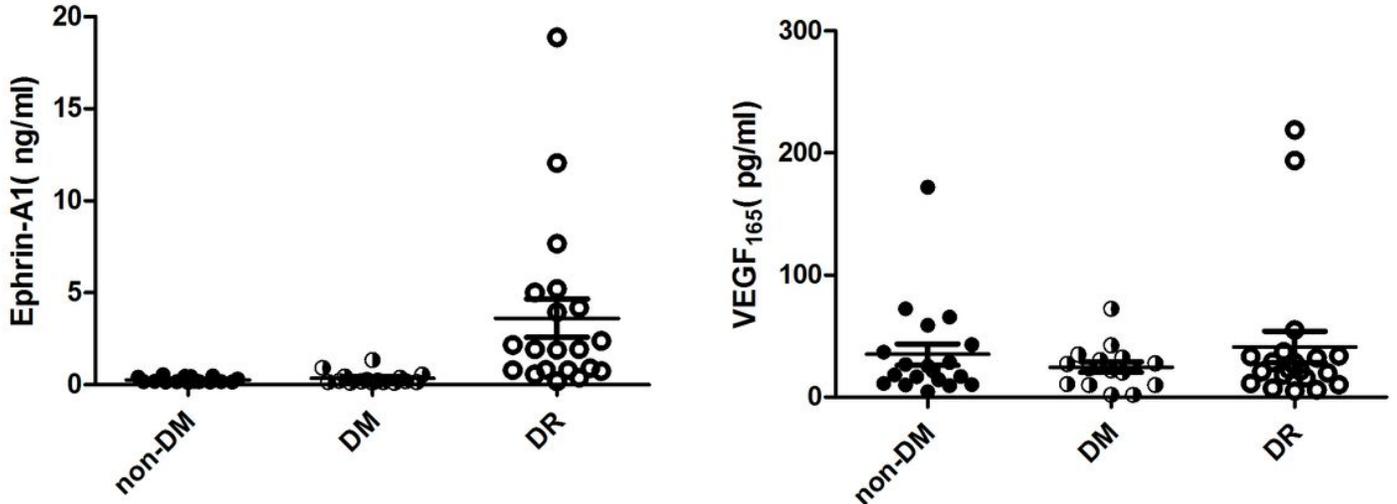


Figure 1

Plasma VEGF165 and Ephrin-A1 concentrations in DR and non-DR subjects.