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Serum CD73 and apelin levels in patients with diabetic retinopathy and the clinical significance

Wei−Qiang Du✉

Clinical Laboratory Department, People's Hospital of Gaoming District Foshan City Guangdong Province, Foshan City, Guangdong Province, 528500

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ABSTRACT

Objective: To study the serum ecto-5'-nucleotidase (CD73) and apelin levels in patients with diabetic retinopathy (DR) and the clinical significance. **Methods:** A total of 108 patients with type 2 diabetes treated in our hospital between April 2013 and February 2016 were collected and divided into non-diabetic retinopathy (NDR) group (n=51), background diabetic retinopathy (BDR) group (n=40) and proliferative diabetic retinopathy (PDR) group (n=17) based on the results of fundus fluorescence angiography. Enzyme-linked immunosorbent assay (ELISA) was used to determine CD73 and apelin level immediately after admission; thiobarbituric acid method and xanthine oxidase method were used to determine the serum levels of oxidative stress indicators; ELISA method was used to determine the levels of angiogenesis indexes and inflammatory factors; Pearson test was used to analyze the correlation of serum CD73 and apelin levels with the illness-related indexes in patients with DR. **Results:** Serum CD73 and apelin levels of BDR group and PDR group were significantly higher than those of NDR group, and serum CD73 and apelin levels of PDR group were significantly higher than those of BDR group; serum malondialdehyde (MDA), advanced oxidation protein products (AOPP), interleukin-2 (IL-2), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), hypersensitive C-reactive protein (hs-CRP), vascular endothelial growth factor (VEGF), angiogenin-2 (Ang-2) and hypoxia-inducible factor 1α (HIF-1α) levels of BDR group and PDR group were significantly higher than those of NDR group while total antioxidant capacity (TAOC), superoxide dismutase (SOD) and interleukin-10 (IL-10) levels were lower than those of NDR group, and the changes in above indexes of PDR group were more significant; Pearson test showed that serum CD73 and apelin levels in patients with DR were directly correlated with the levels of illness-related indexes. **Conclusion:** CD73 and apelin expression are abnormally high in patients with DR, and their expression is directly correlated with the disease severity, and can be used as the reliable indicators for early diagnosis and prognosis judgment of DR.

1. Introduction

Diabetic retinopathy (DR) is the main performance of the diabetic microangiopathy, belongs to one of the important complications of diabetes, and can be divided into background diabetic retinopathy (BDR) and proliferative diabetic retinopathy (PDR) according to the combination with retinal neovascularization or not[1,2]. If DR is not detected and treated in time, the long-term blindness rate is extremely high, and looking for sensitive serum specific indexes to increase the early diagnostic rate of DR is the focus of current clinical research. Ecto-5′-nucleotidase (CD73) is the key enzyme to generate extracellular adenosine, and research has confirmed its high expression in proliferative membrane specimens of patients with DR, and it is believed to be directly related to DR[3]. Apelin is new adipocytokine, belongs to the angiotensin receptor-related protein and has been confirmed to be involved in vascular steady state, inflammation and multiple other physiological and pathological processes, and some scholars have currently pointed out that it may be involved in the retinal endothelial proliferation and angiogenesis

✉Corresponding author: Wei-Qiang Du, Clinical Laboratory Department, People's Hospital of Gaoming District Foshan City Guangdong Province, Foshan City, Guangdong Province, 528500.
Tel: 0757-88820283; 13318300988
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process[4,5]. In the following study, serum CD73 and apelin levels in patients with diabetic retinopathy and their correlation with inflammation, oxidative stress and angiogenesis were analyzed.

2. Information and methods

2.1 General information

A total of 108 patients with type 2 diabetes treated in our hospital between April 2013 and February 2016 were collected as the research subjects, the patients themselves signed informed consent, and the research was approved by hospital ethics committee. According to the results of fundus fluorescence angiography, the patients were divided into non-diabetic retinopathy (NDR) group (n=51), background diabetic retinopathy (BDR) group (n=40) and proliferative diabetic retinopathy (PDR) group (n=17). Inclusion criteria were as follows: (1) complying with the diagnostic criteria of internal medicine for type 2 diabetes; (2) ≤80 years old; (3) without history of ocular trauma and surgery prior to admission. Exclusion criteria: (1) with glaucoma, choroid detachment, corneal neovascularization and other eye diseases; (2) with severe metabolic syndrome; (3) with severe heart, liver and kidney dysfunction; (4) with malignant tumor diseases; (5) with incomplete clinical data caused by quitting study. NDR group included 30 male cases and 21 female cases, they were 42-75 years old, and the course of diabetes was 5-12 years and 7 years in average; BDR group included 23 male cases and 17 female cases, they were 39-72 years old, and the course of diabetes was 5-11 years and 7 years in average; PDR group included 11 male cases and 6 female cases, they were 45-71 years old, and the course of diabetes was 8-16 years and 8 years in average. The three groups of patients were not statistically different in distribution of gender, age and course of disease (P>0.05).

2.2 Serum index detection methods

2ml of fasting peripheral venous blood was collected from three groups of patients immediately after admission and centrifuged in centrifuge (Sichuan Shuke Instrument Co., LTD., model TGL-2150) at 2 500 r/min centrifuge for 10 min to get supernatant, then ELISA was used to detect CD73, apelin, endothelial growth factor (VEGF), angiogenin-2 (Ang-2), hypoxia-inducible factor 1 α (HIF-1 α), interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis factor- α (TNF- α) and hypersensitive C-reactive protein (hs-CRP levels, thiobarbituric acid method was used to determine malondialdehyde (MDA), total antioxidant capacity (TAOC) and superoxide dismutase (SOD), and xanthine oxidase method was used to determine advanced oxidation protein products (AOPP) levels.

2.3 Statistical methods

Data in the study was input in software SPSS 20.0, measurement data was in terms of Mean ± SD, comparison among three groups was by variance analysis, comparison between two groups was by t test, correlation analysis was by Pearson test and P<0.05 indicated statistical significance in differences.

3. Results

3.1 Serum CD73 and apelin levels

Differences in serum CD73 (pg/mL) and apelin (ng/mL) levels were statistically significant among three groups of patients (P<0.05). Pair-wise comparison between groups showed that serum CD73 and apelin levels of BDR group and PDR group were significantly higher than those of NDR group, serum CD73 and apelin levels of PDR group were significantly higher than those of BDR group, and differences in pair-wise comparison between groups were statistically significant (P<0.05), shown in Table 1.

Table 1.

Comparison of serum CD73 and apelin levels among three groups of patients.

Groups	n	CD73	Apelin
NDR	51	1.17±0.18	4.36±0.57
BDR	40	1.84±0.25*	7.62±0.89*
PDR	17	2.93±0.35*#	11.85±1.76*#
F value		6.492	9.273
P value		<0.05	<0.05

Note: compared with NDR group, *P<0.05; compared with BDR group, #P<0.05.

3.2 Oxidative stress

Differences in serum oxidative stress indexes MDA (mol/L), TAOC (U/mL), SOD (U/mL) and AOPP (ng/mL) levels were statistically significant among three groups of patients (P<0.05). Pair-wise comparison between groups showed that serum oxidation indexes MDA and AOPP levels of BDR group and PDR group were significantly higher than those of NDR group while anti-oxidation indexes TAOC and SOD levels were significantly lower than those of NDR group; serum oxidation indexes MDA and AOPP levels of PDR group were significantly higher than those of BDR group while anti-oxidation indexes TAOC and SOD levels were significantly lower than those of BDR group, and differences in pair-wise comparison between groups were statistically significant (P<0.05), shown in Table 2.

Table 2.

Comparison of serum oxidative stress index levels among three groups of patients.

Groups	n	MDA	TAOC	SOD	AOPP
NDR	51	9.26±0.98	28.36±3.41	101.83±15.47	43.18±5.09
BDR	40	14.17±1.86*	21.76±2.89*	84.38±9.12*	67.92±7.15*
PDR	17	21.09±2.64*#	14.09±1.76*#	60.75±7.01*#	98.55±10.18*#
F value		8.392	8.672	9.162	10.183
P value		<0.05	<0.05	<0.05	<0.05

Note: compared with NDR group, *P<0.05; compared with BDR group, #P<0.05.

3.3 Angiogenesis indexes

Differences in serum angiogenesis indexes VEGF (ng/L), Ang-2 (ng/mL) and HIF-1 α (ng/L) levels were statistically significant among three groups of patients (P<0.05). Pair-wise comparison between groups showed that serum VEGF, Ang-2 and HIF-1 α levels of BDR group and PDR group were significantly higher than those of NDR group; serum VEGF, Ang-2 and HIF-1 α levels of PDR group were

significantly higher than those of BDR group, and differences in pair-wise comparison between groups were statistically significant (P<0.05), shown in Table 3.

Table 3.

Comparison of serum angiogenesis index levels among three groups of patients.

Groups	n	VEGF	Ang-2	HIF-1
NDR	51	50.21±5.88	7.12±0.86	30.27±3.81
BDR	40	97.64±10.07*	9.83±1.05*	48.93±6.13*
PDR	17	145.38±19.64*#	11.16±1.74*#	63.86±7.19*#
F value		11.283	8.394	9.273
P value		<0.05	<0.05	<0.05

Note: compared with NDR group, *P<0.05; compared with BDR group, #P<0.05.

3.4 Inflammatory factors

Differences in serum inflammatory factors IL-2 (ng/L), IL-6 (ng/mL), IL-10 (ng/L), TNF- α (ng/L) and hs-CRP (mg/L) levels were statistically significant among three groups of patients (P<0.05). Pair-wise comparison between groups showed that serum IL-2, IL-6, TNF- α and hs-CRP levels of BDR group and PDR group were significantly higher than those of NDR group while IL-10 levels were significantly lower than that of NDR group; serum IL-2, IL-6, TNF- α and hs-CRP levels of PDR group were significantly higher than those of BDR group while IL-10 level was significantly lower than that of BDR group, and differences in pair-wise comparison between groups were statistically significant (P<0.05), shown in Table 4.

3.5 Correlation analysis

Pearson test showed that serum CD73 and apelin levels in patients with DR were positively correlated with oxidation indexes MDA and AOPP, and negatively correlated with anti-oxidation indexes TAOC and SOD; they were positively correlated with angiogenesis indexes VEGF, Ang-2 and HIF-1 α levels; and they were positively correlated with inflammatory factors IL-2, IL-6, TNF- α and hs-CRP levels, and negatively correlated with IL-10 level (P<0.05).

4. Discussion

DR is the most common ocular vascular complication in diabetic patients with poorly controlled blood glucose, and it is also one of the main clinical blinding eye diseases[6]. Fundus examination is the gold standard for diagnosis of DR, but it is unable to be popularized in daily inspection, which causes that many patients with DR are clinically diagnosed until vision loss[7]. Serologic test is the most easily popularized method for DR screening, and looking for the serological indicators with high sensitivity and specificity is the focus of current DR research. CD73 has been found to participate in the process of tumor angiogenesis, and the number of tumor angiogenesis sharply reduces in breast cancer model mice with CD73 gene knockout. CD73 degradation product adenosine has pro-angiogenesis effect, can promote retinal vascular endothelial cell proliferation and lumen formation, and plays an important role in the DR[8]. Apelin, as a adipocytokine, plays an important role in immune regulation and glucose homeostasis maintenance, and the study of PENG An-lin[9] confirms that Apelin can strengthen the retinal endothelial cell migration and proliferation as well as capillary angiogenesis, and is thought to participate in the angiogenesis process of DR. Detection of serum CD73 and apelin levels in DR patients with different conditions in the study showed that compared with the NDR group, BDR group and PDR group were with higher serum CD73 and apelin levels, and with the aggravation of DR condition, serum CD73 and apelin levels further increased, it shows that abnormally highly expressed CD73 and apelin are directly involved in the development of DR, but their inner link with the specific DR severity needs to be confirmed by further research.

There is significant systemic oxidative stress in patients with sustained high blood glucose, and it is also one of the internal mechanisms of DR development[10]. Non-enzymatic advanced glycation end-products of proteins and oxygen free radicals will be constantly produced in patients with DR under high glucose state, which increase retinal ischemic hypoxic injury and cause retinal capillary micro-thrombosis[11,12]. The degree of oxidative stress in patients with DR is directly positively correlated with the severity of retinopathy, so serum levels of oxidation and anti-oxidation factors of all groups were detected in the study, and it was found that compared with the NDR group, BDR group and PDR group were with higher serum oxidation indexes MDA and AOPP levels, and lower anti-oxidation indexes TAOC and SOD levels, and the oxidation/anti-oxidation imbalance is intensified with the aggravation of DR.

Angiogenesis and massive expression of pro-angiogenesis factors is the main performance in the proliferative period of DR, and also the visual symbol of DR severity, so the detection of serum angiogenesis indexes in patients with DR can objectively reflect the specific illness[13,14]. Vascular endothelial growth factor (VEGF), angiogenin-2 (Ang-2) and hypoxia-inducible factor 1 α (HIF-1 α) are the currently recognized pro-angiogenesis factors, VEGF is with the strongest pro-angiogenesis ability, HIF-1 α is specifically produced in retinal ischemic hypoxic state and

Table 4.

Comparison of serum inflammatory factor levels among three groups of patients.

Groups	n	IL-2	IL-6	IL-10	TNF- α	hs-CRP
NDR	51	63.27±7.81	3.42±0.45	65.38±7.12	50.23±5.98	1.62±0.18
BDR	40	81.94±8.74*	5.76±0.66*	50.21±5.48*	78.92±8.17*	3.04±0.35*
PDR	17	105.37±18.93*#	9.17±0.98*#	34.26±4.09*#	90.25±9.76*#	6.71±0.78*#
F value		9.283	7.821	8.262	8.617	7.273
P value		<0.05	<0.05	<0.05	<0.05	<0.05

Note: compared with NDR group, *P<0.05; compared with BDR group, #P<0.05.

enhances the transcriptional activity of VEGF, and Ang-2 content is consistent with the VEGF content[15,16]. It was found in the study that compared with NDR group, BDR group and PDR group were with higher serum VEGF, Ang-2 and HIF-1 α levels, which are basically consistent with the research results of WANG Yi[17]; Further comparison between DR patients with different conditions showed that serum VEGF, Ang-2 and HIF-1 α levels further increased with the aggravation of DR, confirming that angiogenesis indexes play an important role in the DR progression, and also showing that their levels can accurately determine the severity of DR. There is micro-inflammation state in diabetic patients, high blood glucose and oxidative stress state can persistently increase the body's inflammatory state, and meantime, the retina will release more inflammatory cytokines and stimulate angiogenesis in anoxic condition, and form a vicious cycle together with oxidative stress and other reactions[18,19]. In the study, serum inflammatory factor levels of all groups were detected, and it was found that pro-inflammatory factors IL-2, IL-6, TNF- α and hs-CRP levels of BDR group and PDR group were significantly higher than those of NDR group while anti-inflammatory factor IL-10 levels were significantly lower than that of NDR group; and the changes in above pro-inflammatory and anti-inflammatory factor levels of PDR group were more significant. In order to determine the value of serum CD73 and apelin levels for DR condition judgment, the Pearson test was adopted in this study to evaluate the correlation of serum CD73 and apelin levels in DR patients with the disease severity indexes mentioned above, and it was found that serum CD73 and apelin levels in patients with DR were positively correlated with oxidation indexes MDA and AOPP, and negatively correlated with anti-oxidation indexes TAOC and SOD; they were positively correlated with angiogenesis indexes VEGF, Ang-2 and HIF-1 α levels; and they were positively correlated with inflammatory factors IL-2, IL-6, TNF- α and hs-CRP levels, and negatively correlated with IL-10 level. To sum up, it is concluded as follows: CD73 and apelin expression are abnormally high in patients with DR, their expression is directly correlated with the disease severity, they can be used as the reliable indicators for early diagnosis and prognosis judgment of DR, and they are worthy of popularization and application in clinical practice in the future.

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