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Significance of change of retinol binding protein 4 level of plasma of patients with coronary heart disease complicated with hyperlipidemia

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Abstract. – OBJECTIVE: We investigated the significance of a change in retinol binding protein (RBP4) levels in the plasma of patients with coronary heart disease complicated with hyperlipidemia.

PATIENTS AND METHODS: 66 cases of coronary heart disease (CHD) patients in Cardiovascular Department of our Hospital from December 2014 to December 2015 were selected. Based on the diagnostic criteria of hyperinsulinemia, patients were divided into a CHD Group with 35 cases and a CHD-Hyperinsulinemia Group (CHD-H group) with 31 cases. 35 healthy adults who had a physical examination in our hospital medical center during the same period were selected as the control group.

RESULTS: Changes in fasting plasma glucose (FPG), uric acid (UA), high sensitive C-reactive protein (hs-CRP), free fatty acid (FFA), triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), insulin, RBP4 and HOMA-IR were measured in the plasma of the subjects of the 3 groups, and the correlation of RBP4 levels and other indicators were analyzed. UA, hs-CRP, TG and HDL-C in the CHD group and the CHD-H group were significantly higher than those in the control group ($p<0.05$), in which the UA level of CHD-H group was higher than that of CHD group ($p<0.05$). The level of LDL-C in CHD group was higher than that in control group ($p<0.05$); FIns, 2h PIns, HOMA-IR and RBP4 of CHD-H group were significantly higher than those of the control group and CHD group ($p<0.05$); 2h PIns and HOMA-IR are independent related factors of RBP4.

CONCLUSIONS: The level of BRP4 in plasma of CHD patients with high insulin was increased. BRP4 was also found to participate in the occurrence of insulin resistance, which may indicate its role as a potential biomarker which identifies cases of insulin resistance of CHD patients and is worth further study.

Key Words:
Coronary heart disease, Hyperinsulinemia, Plasma retinol-binding protein 4, Atherosclerosis.

Abbreviations

CHD = coronary heart disease; UA = uric acid; FPG = fasting plasma glucose; hs-CRP = high sensitive C-reactive protein; FFA = free fatty acids; HOMA-IR = Homeostasis Model Assessment of Insulin Resistance, FIns = fasting insulin; 2h PIns = 2h postprandial insulin;

Introduction

Coronary heart disease (CHD) is a kind of heart disease caused by coronary artery stenosis, which leads to vascular stenosis or obstruction, resulting in myocardial ischemia, hypoxia and even necrosis[1]. Often referred as "coronary heart disease", CHD is a great danger to the health of a human subject being with urgent and rapid development, high incidence and high mortality[2]. The main risk factors of CHD include hypertension, glucose, lipid metabolism disorders, obesity, smoking and a lack of exercise[3]. Studies have found that the risk of the CHD patients with perennial disease is 3-3.5 times of people with normal blood glucose[4]. The clinical data of our country also showed that patients with abnormal glucose metabolism in patients with CHD accounted for about 76% of the CHD population[5]. Epidemiological data show that the incidence and mortality of CHD worldwide is on the rise[6], and as the people's diet structure changes, we must also change our focus to the patients with abnormal metabolism of glucose. This study investigated the significance of the change in levels of retinol

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binding protein (RBP4) in the plasma of patients with coronary heart disease complicated with hyperlipidemia.

Patients and Methods

Patients

A total of 66 cases of CHD patients in the Cardiovascular Department of our hospital from December 2014 to December 2015 were selected. There were 36 males and 30 females, aged 44-74 years old. Inclusion criteria: (1) CHD diagnostic criteria (7): coronary artery stenosis >50% was found by coronary angiography; (2) Diagnostic criteria for the diagnosis of hyperlipidemia (8): fasting insulin, Fins ≥15 mU/L, and/or 2 hours postprandial insulin, 2h PIns ≥80 mU/L; (3) Patients and their families understand the research program and were willing to accept the relevant checks. Exclusion criteria: (1) Patients with diabetes mellitus or impaired glucose tolerance; (2) Myocardial infarction or cerebral vascular accident occurred within 3 months; (3) Liver and kidney dysfunction; (4) Recent severe infection with immune dysfunction; (5) Malignant tumor.

Based on the diagnostic criteria of hyperinsulinemia, patients were divided into a CHD Group with 35 cases and a CHD-hyperinsulinemia Group (CHD-H group) with 31 cases. Among the CHD group, 19 cases were male, 16 cases were female, aged (63.71 ± 9.64) years old. In the CHD-H group, 17 cases were male, 14 cases were female, aged (63.57 ± 8.71) years old, 35 healthy adults who had physical examination in our hospital medical center during the same period were selected as the control group, among which 18 males, 17 females, aged (62.72±6.71) years old, and there was no statistically significant difference in the general data such as the ratio of sex, age, BMI of three groups.

Instruments and Reagents

Ultra low temperature refrigerator was purchased from Thermo Fisher (Waltham, MA, USA), low temperature and high-speed centrifuge was purchased from Thermo Fisher (Waltham, MA, USA), the automatic biochemical analyzer was purchased from Beckman Coulter Company (Fullerton, CA, USA), the enzyme standard instrument was purchased from Bio-Rad (Hercules, CA, USA). Human insulin and human RBP4 test kit were purchased from Abbott (Abbott Park, IL, USA).

Specimen Collection and Detection

5 ml fasting venous blood of all subjects was collected in the early morning and injected into the anticoagulant tube, 3000 rpm centrifugal 10 min, collect plasma, after packaging it, store it in ultra-low temperature refrigerator. The research subjects ate 100 g buns or bread (equivalent to glucose 75 g) and, after 2h, the blood was collected with the same method and the blood serum was preserved A full automatic biochemical analyzer was used to measure fasting plasma glucose (FPG), uric acids (UA), high-sensitive C-reactive protein (hs-CRP), free fatty acids (FFA), triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) of the plasma. The insulin and RBP4 levels in plasma were detected by Enzyme-Linked ImmunoSorbent Assay, ELISA, and the operation was carried out in strict accordance with the specification. Calculate Homeostasis model assessment-insulin resistance, HOMA-IR=FPG×FIns/22.5.

Statistical Analysis

All data was analyzed by SPSS 17.0 (SPSS Inc., Chicago, IL, USA), measurement data were expressed by $\bar{x} \pm s$, group comparison was analyzed by single factor variance analysis, count data were tested by χ^2, index correlation was analyzed by Pearson correlation. $p < 0.05$ indicated that differences have statistical significance.

Results

Comparison of General Blood Biochemical Indexes

UA, hs-CRP, TG and HDL-C in CHD group and CHD-H group were significantly higher than those in control group ($p<0.05$), in which the UA level of CHD-H group was higher than that of CHD group ($p<0.05$), the level of LDL-C in CHD group was higher than that in control group (Table I) ($p<0.05$).

Insulin and RBP4 Level

FIns, 2h PIns, HOMA-IR and RBP4 of CHD-H group were significantly higher than those of the control group and the CHD group and the difference was statistically significant (Table II) ($p<0.05$).

Correlation between Plasma RBP4 and Various Indexes

FPG, UA, hs-CRP, TG, FIns, 2h PIns, HOMA-IR and RBP4 were positively correlated

Table I. Comparison of blood biochemical indexes of the three groups.

Index	Control group (n=35)	CHD group (n=35)	CHD-H group (n=31)	F	p
FPG (mmol/L)	4.73±0.42	4.81±0.48	5.02±0.57	2.631	0.236
UA (μmol/L)	278.42±64.23	335.04±75.81*	380.01±94.92*#	11.325	0.001
hs-CRP (mg/L)	0.32±0.15	3.47±3.98*	7.01±6.59*	8.647	0.004
FFA (mmol/L)	0.54±0.14	0.59±0.31	0.56±0.42	0.782	0.654
TG (mmol/L)	0.84±0.29	1.34±0.82*	1.62±0.66*	5.271	0.027
TC (mmol/L)	4.41±0.66	4.35±1.36	4.07±1.01	1.761	0.327
LDL-C (mmol/L)	1.90±0.27	2.42±0.23*	2.06±0.34	4.668	0.013
HDL-C (mmol/L)	2.07±0.66	1.24±0.27*	1.22±0.28*	5.281	0.009

Note: compared with the control group, *$p<0.05$; Compared with CHD group, #$p<0.05$

Table II. Insulin and RBP-4 levels in three groups.

Index	Control group (n=35)	CHD group (n=35)	CHD-H group (n=31)	F	p
FIns (mU/L)	4.76±1.62	6.21±2.48	19.42±4.37*#	10.281	0.001
2hPIns (mU/L)	28.32±6.23	29.04±7.81	95.41±14.62*#	14.342	0.000
HOMA-IR	0.92±0.22	1.47±0.48	2.41±0.59*#	6.317	0.007
RBP4 (mg/L)	16.54±4.34	18.39±6.61	27.36±8.32*#	9.782	0.003

Note: compared with the control group, *$p<0.05$; Compared with CHD group, #$p<0.05$

($p<0.05$), HDL-C and RBP4 were negatively correlated ($p<0.05$). We took the indicators above as the independent variables, RBP4 as the dependent variable, and take progressive regression analysis, and the result is that 2h PIns ($r^2=0.192$, $p<0.05$) and HOMA-IR ($r^2=0.282$, $p<0.05$) are the independent related factors of RBP4 (Table III).

Discussion

CHD is a disease that significantly endangers human health. In addition to causing death, CHD often leads to loss of the labor force, and brings huge economic burden to the family[9]. According to the statistical data of residents of 8 cities, the average annual economic burden of disease of CHD is as high as renminbi (RMB) 7825, among which the cost of hospitalization accounted for more than 50%[10]. Another study in the United States shows that the direct economic losses generated by CHD is up to $21406[11]. Due to the high cost of treatment and a heavy economic burden, many CHD patients choose to give up treatment or resist treatment, resulting in greater harm to patients and families. The pathogenesis of CHD has not been fully elucidated and many factors can induce CHD, but it is now clear that atherosclerosis (AS) is the pathological basis of CHD[12,13].

Table III. Correlation between RBP4 and various indexes.

Index	R	P
FPG	0.313	0.002
UA	0.234	0.022
hs-CRP	0.223	0.028
FFA	0.067	0.467
TG	0.260	0.026
TC	0.242	0.274
LDL-C	0.010	0.918
HDL-C	-0.310	0.006
FIns	0.592	0.001
2h PIns	0.542	0.001
HOMA-IR	0.612	0.001

RBP4, a new fat cells factor first discovered in 2005 by the research team of Harvard University, is a retinol binding protein family member. The gene is located on the long arm of chromosome 10. RBP4 is a secreted protein, predominantly involved in the binding and transport of vitamin A[14,15]. RBP4 can also bind retinol, form soluble complexes, protect it from the specific oxidation and reduce the toxic effect. In addition, it is also involved in the growth and differentiation of epithelial tissue and bone tissue[16]. Some works

have found that RBP4 is one of the risk factors for atherosclerosis[17]; studies have also indicated that circulating RBP4 levels are associated with insulin resistance, diabetes, and other diseases[18].

This study showed that FIns, 2h PIns, HOMA-IR and RBP4 of CHD-Hyperinsulinemia patients were significantly higher than those of the control group and CHD group, the difference between CHD patients and healthy adults was not statistically significant. Correlation analysis showed that RBP4 was significantly correlated with the indicators above. Regression analysis suggested that 2h PIns and HOMA-IR were independent related factors of RBP4. Combining results from previous studies and the results of this investigation, the possible reasons are, firstly, that RBP4 is closely related to a variety of risk factors for atherosclerosis, such as hs-CRP, TG and HDL-C found in this study, among which the increase of cholesterol is an important risk factor for the occurrence of AS[19]. The increase of hs-CRP indicates the presence of inflammation in the body, and the inflammatory state will aggravate the endothelial damage and lead to vascular intimal injury[20]; HDL-C is an important indicator of resistance to atherosclerosis, and it is the protective factor of CHD[21]. These results suggest that RBP4 plays a role in the promotion of atherosclerosis occurrence and development, and high level of insulin is also proved to be a risk factor of AS by research, thereby establishing a relationship between RBP4 and the resistance to high insulin levels. Secondly, the "common soil theory" proposed by Stern[22] that insulin resistance is the basic pathological change of many diseases, the abnormal expression of BRP4 protein is an important part of insulin resistance and epidemiological investigations and animal experiments have shown that RBP4 prevents phosphorylation of the insulin receptor, and increases the sensitivity of insulin receptor[23].

Conclusions

The level of BRP4 of plasma of CHD patients with high insulin was increased. In addition, BRP4 also participates in the occurrence of insulin resistance, which may be the biomarker which identifies the insulin resistance of CHD patients that is worth further study.

Conflicts of interest

The authors declare no conflicts of interest.

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