The Association of Paraoxonase-3 and Glycoxidative Apolipoprotein A-I with Diabetes Mellitus Complicated with Coronary Artery Disease

Xia Yang

Department of Cardiovascular Medicine, NO.101 Hospital of PLA, Wuxi, Jiangsu 214044, China

Abstract: Objective: To explore the association of serum paraoxonase-3 (PON3) and glycoxidative apolipoprotein A-I (ApoA-I) levels with diabetes mellitus (DM) complicated with coronary artery disease (CAD). **Methods:** 90 DM patients who were in NO.101 Hospital of PLA from January, 2012 to December, 2014 were selected and divided into DM without CAD group (n=48) and DM with CAD group (n=42). 50 healthy subjects were served as control group. Serum PON3 levels and glycoxidative apoA-I levels were measured to analyze their association with DM complicated with CAD. **Results**: Serum PON3 levels were significantly lower in DM with CAD group and DM without CAD group than those in control group, while the glycoxidative apoA-I levels were significantly higher than control group. There were statistical differences (P<0.05). Serum PON3 levels were significantly lower in DM with CAD group than those in DM without CAD group, while the glycoxidative apoA-I levels were higher than those in DM without CAD group. There were statistical differences (P<0.05). In DM with CAD group, PON3 levels in patients with multi-vessel disease were significantly lower than those in patients with 1-vessel disease and patients with 2-vessel disease, while the glycoxidative apoA-I levels of patients with multi-vessel disease were significantly higher than those of patients with 1-vessel disease and patients with 2-vessel disease. There were statistical differences (P<0.05). Serum PON3 level was negatively correlated with glycoxidative apoA-I level and the number of diseased coronary vessels (r=-0.217, r=-0.177 respectively, all P<0.05). Glycoxidative apoA-I level was positively correlated with the number of diseased coronary vessels (r=0.713, P<0.05). PON3 and glycoxidative apoA-I were independent predictive factors of DM complicated with CAD (all P<0.01). **Conclusion:** Serum PON3 in patients with DM complicated with CAD presents lower expression while glycoxidative apoA-I present higher expression, and they are in negative correlation. PON3 and glycoxidative apoA-I are closely associated with DM complicated with CAD.

[Xia Yang. **The Association of Paraoxonase-3 and Glycoxidative Apolipoprotein A-I with Diabetes Mellitus Complicated with Coronary Artery Disease.** *Life Sci J* 2015;12(4):184-188]. (ISSN:1097-8135). http://www.lifesciencesite.com. 23

Key words: PON3; glycoxidative ApoA-I; DM; CAD

Introduction

Diabetes mellitus (DM) is a common internal medical disease, and has high morbidity. It is usually complicated with heart, brain and kidney disease, especially with coronary artery disease (CAD), which has important effects on patients' health [1-3]. With the constant research, researchers have found that the expression of ApoA-I has close relation with DM complicated with CAD. Apolipopretein lysine residues and reactive carbonyl form glycoxidase Nε-carboxy methyl lysine (CML) after a serial reaction, and it can easily result in the afunction of high-density lipoprotein (HDL) [4]. Under the normal physiological condition, HDL is exposed to high levels of reactive oxygen species of macrophage. ApoA-I which is the most important structural protein of HDL is closely combined with enough PON3 to protect it from oxidation. But there is still no report about whether the low level of PON3 can't protect ApoA-I from oxidation and thus prompts the development of DM complicated CAD. This research mainly explored the association of PON3 and glycoxidative ApoA-I with DM complicated with CAD.

1. Materials and methods

1.1 Materials

90 type 2 diabetes mellitus (T2DM) patients who were in NO.101 Hospital of PLA from January, 2012 to December, 2014 were selected and divided into DM without CAD group (n=48) and DM with CAD group (n=42). 50 healthy subjects were served as control group. There were 27 males and 21 females in DM without CAD group, aged from 44 to 83 years old (average 65.36 ±10.83). There were 25 males and 17 females in DM with CAD group, aged from 45 to 86 years old (average 66.54±11.51), and 11 patients with 1-vessel disease, 16 patients with 2-vessel disease, 15 patients with multi-vessel disease. There were 28 males and 22 females in control group, aged from 42 to 78 years old (average 62.21±8.48).

According to standards of American DM association, T2DM is diagnosed by blood sugar concentration with random blood sugar concentration above 11.1mmol/L or fasting blood sugar concentration above 7.0mmol/L or sugar blood concentration above 11.1mmol/L 2 hours after dinner.

CAD diagnosis standard: lumen stenosis degree of left main coronary artery, anterior descending branch, circumflex branch or right main coronary artery in coronary angiogram is above 50.0%. 1-vessel disease in coronary artery lesion is mainly concerned with one of the left anterior descending branch, left circumflex branch or the right coronary artery. 2-vessel disease is concerned with any two of these three branches, and multi-vessel disease is concerned with three or above three coronary arteries.

1.2 Methods

1.2.1 Regular indicator detection

Fasted more than 8 hours and extracted the elbow venous blood to detect blood lipid, glycosylated hemoglobin (HbA1c) and high-sensitivity C-reactive protein (hs-CRP) of patients. Diagnosis standards of hyperlipidaemia (HLP): ①Serum LDL-C>3.64mmol/L; ②Serum HDL-C<0.91mmol/L; ③Serum TG> 1.70mmol/L; ④Serum TC>5.72mmol/L. Conforming to any of these four standards can be diagnosed as HLP. Blood pressure of patients in these three groups was detected and incidence rate of hypertension was calculated. Diagnosis standard of hypertension: systolic pressure≥140mmHg and/or diastolic pressure≥ 90mmHg.

1.2.2 PON3 detection

ELISA method was used to measure the expression level of PON3, kits were provided by Japanese Xiehe Pharmaceutical co., LTD and linearity range was from 0.156ng/mL to 10.000ng/mL, and the maximum coefficient of variation of inter-assay was less than 3.0%.

1.2.3 Glycoxidative ApoA-I detection

HDL was obtained by ultracentrifugation of whole blood, 10% sodium dodecyl sulfate - polyacrylamide gels (SDS-PAGE) was used for electrophoresis separation of ApoA-I, then the Amersham ECL Western blotting detection reagents and analysis system (GE Healthcare, UK) were used to detect protein bands, HP flatbed scanner (Scan jet Pro, USA) conducted a comprehensive analysis of the scan, Adobe Photoshop CS2 software was applied to quantitatively analyze the scanning strip. Absolute density of protein was the product of gray value of immunoblot strips times the pixel, glycoxidative ApoA-I levels were expressed by relative density (absolute density of glycoxidative ApoA-I / absolute density of ApoA-I) [5].

1.3 Statistical analysis

SPSS 18.0 was used to analyze and handle data, percentage (%) was used to express the enumeration data and chi-square test was used for data analysis. Measurement data were expressed by mean value ± standard deviation ($\bar{x}\pm s$) and analyzed by one-way ANOVA. Sperman's method was adopted to analyze the association of serum PON3 and glycoxidative ApoA-I with the number of diseased coronary arteries. Variable which is statistically significant in single factor analysis was brought to multi-factor Logistic regression analysis to adjust confounding factors, thus to identify whether PON3 level and glycoxidative ApoA-I level are independent risk factors for DM complicated with CAD. If P<0.05, the difference is statistically significant.

2. Results

2.1 General information of patients

There were no statistically significant differences in age, gender and smoking rate among these three groups (P>0.05), while there were statistically significant differences in incidence rate of hypertension, HLP, HbA1c level and hs-CRP level (P<0.05) (See Table 1).

Table 1. Comparison of the general information of patients in three groups

Indicators	DM with CAD group	DM without CAD group	Control group	*P* value
Age($\bar{x}\pm s$)	66.54±11.51	65.36±10.83	62.21±8.48	0.11
Male (n, %)	25(59.52)	27(56.25)	28(56.00)	0.93
Smoking patients (n, %)	10(23.80)	9(18.75)	7(14.00)	0.48
Hypertension patients (n, %)	32(76.19)	23(47.92)	0	＜0.05
HLP (n, %)	26(61.90)	21(43.75)	0	＜0.05
HbA1c (%)	8.41±1.90	7.93±1.55	4.65±0.74	＜0.05
hs-CRP (mg/L, $\bar{x}\pm s$)	9.86±6.72	6.34±3.38	2.79±1.51	＜0.05

2.2 Expression levels of serum PON3 and glycoxidative ApoA-I in three groups

Serum PON3 levels were significantly lower in DM with CAD group and DM without CAD group than those in control group, while the glycoxidative ApoA-I levels were significantly higher than control group. There were statistical differences (P<0.05). Serum PON3 levels were significantly lower in DM

with CAD group than those in DM without CAD group, while the glycoxidative ApoA-I levels were higher than those in DM without CAD group. There were statistical differences (P<0.05) (See Table 2).

Table 2. Comparison of Serum PON3 and glycoxidative ApoA-I expression level in three groups

Indicators	DM with CAD group	DM without CAD group	Control group
PON3(ng/mL)	1.44±0.11[ab]	1.97±0.15[a]	2.21±0.51
Glycoxidative ApoA-I (relative density)	6.71±1.14[ab]	4.11±1.03[a]	1.04±0.09

Compared with control group, [a] $P<0.05$; compared with DM without CAD group, [b] $P<0.05$.

2.3 Expression levels of PON3 and glycoxidative ApoA-I in DM with CAD group concerned with the number of diseased coronary arteries

PON3 levels in patients with multi-vessel disease were significantly lower than those in patients with 1-vessel disease and 2-vessel disease, while the glycoxidative ApoA-I levels of patients with multi-vessel disease were significantly higher than those of patients with 1-vessel disease and 2-vessel disease. There were statistical differences (P<0.05). PON3 levels in patients with 2-vessel disease were significantly lower than those in patients with 1-vessel disease, while the glycoxidative ApoA-I levels of patients with 2-vessel disease were significantly higher than those in patients with 1-vessel disease (P<0.05) (See Table 3).

Table 3. Comparison of expression levels of PON3 and glycoxidative apoA-I in DM with CAD group concerned with the number of diseased coronary arteries ($\bar{x}\pm s$)

Indicators	1-vessel disease	2-vessel disease	Multi-vessel disease
PON3(ng/mL)	1.77±0.13[a]	1.46±0.17[ab]	1.21±0.62
Glycoxidative ApoA-I (relative density)	5.21±1.02[a]	6.42±1.13[ab]	8.41±2.03

Compared with multi-vessel disease, [a] $P<0.05$; compared with 1-vessel disease, [b] $P<0.05$.

2.4 Immunoblotting analysis of ApoA-I levels and glycoxidation levels

Glycoxidative ApoA-I levels in DM with CAD group were significantly higher than those in DM without CAD group and control group (See Figure 1 and Table 2).

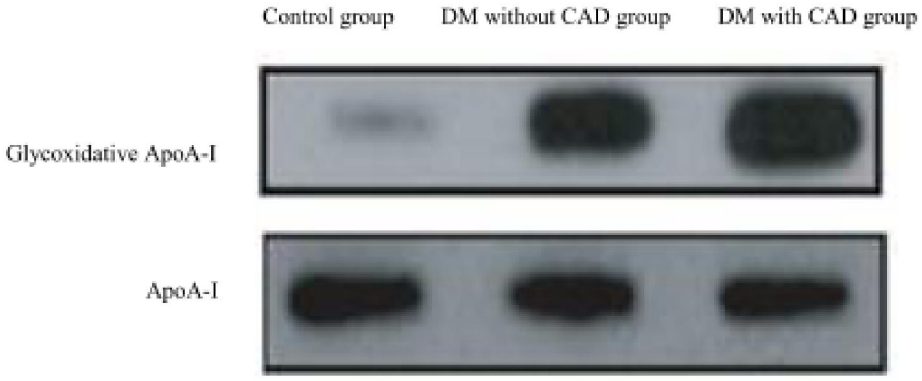

Figure 1. Immunoblotting figure of apoA-I levels and glycoxidation levels

2.5 The correlation of PON3 and glycoxidative ApoA-I with the number of diseased coronary arteries

Serum PON3 level was negatively correlated with glycoxidative ApoA-I level (Pearson's, r=-0.271, $P<0.05$) and the number of diseased coronary arteries (Pearson's, r=-0.177, P<0.05). Glycoxidative ApoA-I level was positively correlated with the number of diseased coronary arteries (Pearson's, r=0.713, P<0.05).

2.6 Multi-factors Logistic regression analysis of DM complicated with CAD

Results show that PON3 and glycoxidation ApoA-I were independent predictive factors of DM complicated with CAD (P<0.01) (See Table 4).

Table 4. Logistic regression analysis of the risk factors in DM complicated with CAD group

Risk factors	OR	95.0% confidence interval	P
PON3	0.771	0.422-1.433	0.004
Glycoxidative ApoA-I	1.784	1.123-2.631	0.001
hypertension	5.651	2.447-9.596	0.001
HLP	3.172	1.624-5.241	0.001
HbA1c	1.348	0.715-1.872	0.002
hs-CRP	1.277	0.834-1.719	0.002

3. Discussion

DM complicated with CAD is a common clinical disease, which has high morbidity and an important impact on patients' health. Therefore, it is significant to know more about DM complicated with CAD in clinic. In order to further know the disease, this study analyzed levels of serum PON3 and glycoxidative ApoA-I, and also found out their correlation with DM complicated CAD [6-7].

This study indicated that PON3 level in DM with CAD group was obviously lower than that in DM without CAD group and control group, but the glycoxidative ApoA-I level was the opposite, which suggested that PON3 and glycoxidative ApoA-I may play certain roles in the pathogenesis of DM complicated with CAD[8]. By analyzing the serum PON3 and glycoxidative ApoA-I in patients with different degrees of coronary artery lesions in DM with CAD group, we found that PON3 levels in patients with multi-vessel disease were obviously lower than that in patients with 2-vessel disease and 1-vessel disease, and PON3 levels in patients with 2-vessel disease were lower than that in patients with 1-vessel disease. However, the glycoxidative ApoA-I levels were the opposite. These data further explained that PON3 and glycoxidative apoA-I may play key roles in the development of DM complicated with CAD. Some clinical studies have showed that glycoxidation modification of apoA-I lysine residues plays a key role in the development of atherosclerosis of DM patients [9-10]. By analyzing the risk factors of DM complicated with CAD, we found that PON3, glycoxidative ApoA-I, HLP, HbA1c and hs-CRP are all risk factors[11]. It further explains that glycoxidation modification of ApoA-I lysine residues is mainly affected by blood sugar, which is consistent with the previous clinical studies[12]. The Study showed that inflammatory cytokines C-reactive protein is closely related to atherosclerosis of CAD patients, and is independent of lipid peroxidation, so it is probably related to glycoxidative ApoA-I[13]. Moreover, Serum PON3 level was negatively correlated with glycoxidative ApoA-I level and the number of diseased coronary vessels while the glycoxidative ApoA-I level was positively correlated with the number of diseased coronary vessels [14]. It further explains that low PON3 level can't protect apoA-I from oxidation, which makes apoA-I lysine residues generate carboxy methyllysine under glycoxidation modification and incurs CAD. PON3 can protect circulating lipoproteins from oxidation, and further regulates the sensitivity of HDL to glycoxidation modification. At the same time, detecting glycoxidative ApoA-I can better predict the risk situation of DM complicated with CAD, and further know coronary lesion degree of patients with CAD [15].

In conclusion, serum PON3 of DM complicated with CAD patients present low expression in clinic, glycoxidative ApoA-I present high expression, and they are in negative correlation. Meanwhile, glycooxidative ApoA-I has a close relationship with DM complicated with CAD.

References

[1] Mo Zushuang. Clinical Analysis of Dangerous Factors of Type 2 Diabetes Amalgamated Coronary Heart Disease [J]. Diabetes World, 2013, 7(10):456-458.

[2] Wang Jingjing, Ding jiang. Risk Factors and Coronary Artery Lesion Characteristics Analysis of Patients with Coronary Heart Disease Complicated with Type 2 Diabetes Mellitus [J]. Journal of Ningxia Medical University, 2010, 32(04):501-503.

[3] Zhan Meien, Chen Kai, Tang Wenxin, et al. Clinical Study on the Risk Factors and Coronary Features of Patients With Premature Coronary heart Disease [J]. Progress in Modern Biomedicine, 2013, 13(06): 1137-1141.

[4] Schweikert E.M., Devarajan A., Witte I., et al. PON3 is upregulated in cancer tissues and protects against mitochondrial superoxide-mediated cell death [J]. Cell Death Differ, 2012, 19(9):1549-1560.

[5] Li Fashun, Wang Tingfa, Zhang Youren. Clinical Analysis of the Lipid, Apolipoprotein A-1 and Apolipoprotein B Checking for Patients with Coronary Heart Disease [J]. China Continuing Medical Education, 2014, 2(05):90-91.

[6] Rao Rong, Liu Zhijun. Analysis of the Serum Lipoprotein (a) and Apolipoprotein A-1, Apolipoprotein B Checking for Patients with Coronary Heart Disease [J]. International Journal of Laboratory Medicine, 2011, 32(03):392-393.

[7] Teng Wei, Xue Yongliang, Liu Peng, et al. Correlation of Plasma C-reactive Protein and Apolipoprotein A-I levels with Coronary Artery Disease [J]. Contemporary Medicine, 2012, 18(08):26-27.

[8] Chen Guoliang, Liu Liwei, Xie Shuang, et al. High density Lipoprotein Cholesterol and Its Associate Factors ApoA-I and Serum Amyloid A in Non-diabetic Patients with Coronary Heart Disease[J]. Chinese Journal of Multiple Organ diseases in the Elderly, 2011, 10(01):44-48.

[9] Lu M, Lu Q, Zhang Y, et al. ApoB/ApoA1 is an effective predictor of coronary heart disease risk in overweight and obesity [J]. Journal of biomedical research, 2011, 25(4):266-273.

[10] Hong LF, Yang B, Luo SH, et al. Impact of estimated HDL particle size via the ratio of HDL-C and apoprotein A-I on short-term prognosis of diabetic patients with stable coronary artery disease [J]. J Geriatr Cardiol, 2014, 11(3):245-252.

[11] Belin AC, Ran C, Anvret A,et al. Association of a protective paraoxonase 1 (PON1) polymorphism in Parkinson's disease [J]. Neurosci Lett, 2012, 26, 522(1):30-35.

[12] Yu Xiangdong, Wan Zheng, Zhang Wenjuan, et al. Correlation between Lipoprotein(a) Concentration and Severity of Coronary Artery Disease in Patients with Type 2 Diabetes Mellitus [J]. Tianjin Medical Journal, 2011, 39(10):921-923.

[13] Mashayekhi N.R., Sadrnia S., Chehrei A., et al. The Correlation between Serum ApoA1 and B and Coronary Artery Disease as well as Its Severity [J]. Int Cardiovasc Res J, 2014, 8(1):1-5.

[14] Ghorbanian B., Ravassi A., Kordi M.R., et al. The Effects of Rope Training on Lymphocyte ABCA1 Expression, Plasma ApoA-I and HDL-c in Boy Adolescents [J]. Int J Endocrinol Metab, 2013, 11(2):76-81.

[15] Liu Shanshan, Wang Lu, Qiu Jianping. Correlation of Serum ApoB and ApoA1 Level, ApoA1/ApoB Ratio with Severity of Coronary artery Stenosis [J]. Chinese Journal of Laboratory Diagnosis, 2013, 17(07):1216-1218.

4/9/2015