Original Paper

Correlation between the High Density Lipoprotein and its Subtypes in Coronary Heart Disease

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Key Words

HDL subtypes • CHD • Correlation

Abstract

Background/Aims: To detect the changes of high density lipoprotein (HDL) and its subtypes in serum of patients with coronary heart disease (CHD). **Methods:** 337 hospitalized patients were selected from our hospital during August, 2014 - January, 2015, and divided into CHD group (n = 190) and control group (n = 127). Lipoprint lipoprotein analyzer was used to classify low density lipoprotein (LDL) particle size and its sub-components, as well as HDL particle size and its sub-components. The changes of the subtypes in patients with CHD were statistically analyzed. The possible mechanism was explored. **Results:** (1) Compared with the control group, the concentration of HDL in CHD patients reduced, HDL_L significantly decreased (P < 0.001), while HDL_S increased (P < 0.001); (2) In the patients with HDL less than 1.04 mmol/L among CHD, all HDL subtypes reduced, but HDL_L had the most significant decreased; (3) HDL and all HDL subtypes were positively correlated with apolipoprotein A-I (apoA-I), of which, HDL_L had the biggest correlation with apoA-I (P < 0.001); (4) HDL subtypes had good correlation with HDL, of which, HDL_M had a maximum correlation with HDL (P < 0.001). **Conclusion:** HDL maturation disorders existed in the serum of CHD patients, HDL_L may be protected factor for CHD, whose decrease was closely related with the risk increase of CHD. The cardiovascular protection function of HDL_L may be related with apoA-I content.

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Introduction

Coronary atherosclerosis (AS) is an important pathophysiological mechanism in the development of coronary heart disease (CHD). Studies have confirmed that low density lipoprotein (LDL) is an important risk factor for AS.

According to the particle size, LDL can be divided into A and B types. LDL particles with size larger than 265Å was called big and light LDL, while LDL particles with size smaller than 265Å was called small density-low density lipoprotein (sd-LDL). Studies have demonstrated that the concentration of sd-LDL was positively correlated with the incidence of CHD [1-3].

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A large number of epidemiological studies have demonstrated [4, 5] that high density lipoprotein (HDL) levels are negatively correlated with the incidence of CHD. Thus, HDL has long been known as the "anti-atherosclerotic factor" or "cardiovascular protection factor." However, in clinical work, it is found that although the lipidaemia is standard, the HDL levels are normal or even high, significant vascular residual risk still exist [6, 7]. Therefore, HDL of patients with CHD has not only amount problems, but also quality problems. Improving HDL function may be more important than raising its level.

This topic selected CHD patients with standard LDL and negative sd-LDL. According to the particle size, HDL can be divided into 10 subtypes (HDL₁-HDL₁₀) using lipoprint HDL lipoprotein analyzer, 1-3 types were large particle types (HDL_L), 4-7 types were intermediate particle types (HDL_M), and 8-10 types were small particle types (HDL_S). The study aimed to investigate the changes of HDL and its various subtypes in the serum of patients with CHD, as well as the possible mechanism.

Materials and Methods

Study subjects

Inclusion criteria: In CHD group, the coronary angiography conformed that at least one branch had inner diameter stenosis more than or equal to 50% in the three main coronary arteries [left anterior descending coronary artery (LAD), left circumflex coronary artery (LCX), right coronary artery (RCA)]. In control group, patients with heart disease were excluded by biochemical indicators, chest radiograph, electrocardiogram, echocardiography, coronary angiography and other tests. Exclusion criteria: diseases affected serum lipoproteins such as pulmonary heart disease, pulmonary hypertension, blood disease, severe liver and kidney dysfunction, severe infection and cancer were excluded. 317 hospitalized cases conformed or excluded by coronary angiography were collected continuously from the Second Hospital of Shanxi Medical University during August, 2014 - January, 2015, and divided into CHD group (n = 190, 133 male cases, 57 female cases) and control group (n = 127, 59 male cases, 68 female cases). This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of the Second Hospital of Shanxi Medical University. Written informed consent was obtained from all participants.

Sampling

Instructed the subjects to be fasting for 12h, collected 2ml venous blood in the next morning for 2 tubes, one was sent to laboratory for measuring general indicators such as serum lipid levels including total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), apolipoprotein A (apo-A), apolipoprotein B (apo-B), apolipoprotein E (apo-E), lipoprotein a, blood sugar, liver function, renal function, blood cell analysis, thyroid function; another was performed lipoprotein testing.

LDL subtypes detection

The total blood analyzed by lipoprotein analyzer was centrifuged with 3000 r/min for 10 minutes to separate the serum for use. The Lipoprint LDL Gel tubes were placed on racks after drying, 25 µl serum samples were added to the tubes, then 200 µl stacking gel, performed upside down for several times to mix with the samples, placed under the irradiation light to irradiate for 30 minutes. Poured 1000 ml electrophoresis buffer at the bottom of the electrophoresis tank, 200 ml buffer in the top of the tank, the gel tube was inserted into the hole, picked bubbles in gel tubes with a needle, set electrophoresis current output, for each gel tube, the maximum output voltage was 500 V for 3 mA, started electrophoresis. After about 60 minutes, the electrophoresis was finished, the gel tubes were removed, allowed to stand for 30 minutes but no more than 2 hours. During standing, turned on the scanner to warm up, after finishing standing, the gel tubes were placed on the flatbed inside the scanner according to serial numbers, should not spaced, obtained the images after scanning. The scanned images were analyzed using Lipoprint lipoprotein analyzer software to obtain images and data of the distribution for LDL subtypes. 155 CHD patients with LDL particles larger than 265Å (113 male cases, 42 female cases) and 102 cases (44 male cases, 58 female cases) in the control group were selected for the next step detection for HDL subtypes.

HDL subtypes detection

Took the Lipoprint HDL Gel tubes, drained the buffer on the upper part of the gel, sucked and dried the buffer inside the wall, and dried the outer surface, the treated gel tubes were placed on tube racks. After drying, the Lipoprint LDL gel tubes were placed on gel racks. The 25 μ l serum samples were added to the gel tube, then 300 μ l stacking gel, performed upside down for several times to mix with the samples, put under the irradiation lamp for 30 minutes. After irradiation, poured 1000 ml electrophoresis buffer at the bottom of the electrophoresis tank, 200 ml buffer in the top of the tank, the gel tube was inserted into the hole, picked bubbles in gel tubes with a needle, set electrophoresis current output, for each gel tube, the maximum output voltage was 500 V for 3 mA, started electrophoresis. After about 60 minutes, the electrophoresis was finished, the gel tubes were removed, allowed to stand for 30 minutes but no more than 2 hours. During standing, turned on the scanner to warm up; after finishing standing, the gel tubes were placed on the flatbed inside the scanner according to serial numbers, should not spaced, obtained the images after scanning. The scanned images were analyzed using Lipoprint lipoprotein analyzer software to obtain images and data of the distribution for LDL subtypes.

Statistical analysis

All data analysis was completed using SPSS17.0 statistical software. The quantitative data consistent with the normal distribution were expressed as mean $\pm$ standard deviation. The comparisons between the two groups were performed using t-test. The data among groups were compared using univariate analysis of variance (ANOVA). The rate comparisons between the two groups were performed using χ^2 test. The two factors correlation analysis was performed using bivariate correlation analysis. $P < 0.05$ was considered to be statistically significant.

Results

General information

Compared with the control group, the TC, LDL, body mass index (BMI) of the CHD group increased, the proportion of smoking and male increased, while HDL decreased, the difference was statistics significance ($P < 0.05$; Table 1, 2).

Concentration comparisons among HDL subtypes

Compared with the control group, HDL and HDL_L of the CHD group reduced, while HDL and HDL_S concentration increased (Table 3).

Map results for HDL particles and subtypes between the two groups

Map results for HDL particles and subtypes in the serum of one case in the CHD group and one case in the control group were analyzed and detected by Lipoprint analyzer (Fig. 1, 2).

Table 1. General information comparison between CHD group and control group ($\bar{x} \pm s$)

Index	CHD group (n=155)	Control group (n=102)	T value	P value
Age (years old)	61.52 $\pm$ 9.87	60.03 $\pm$ 9.77	-1.192	0.235
TC (mmol/L)	4.78 $\pm$ 1.09	4.15 $\pm$ 1.09	4.103	<0.001
TG (mmol/L)	1.96 $\pm$ 1.08	1.78 $\pm$ 0.86	0.144	0.886
HDL (mmol/L)	1.08 $\pm$ 0.27	1.22 $\pm$ 0.26	4.293	<0.001
LDL (mmol/L)	2.81 $\pm$ 1.01	2.35 $\pm$ 0.90	3.657	<0.001
apoA-I (mmol/L)	1.24 $\pm$ 0.26	1.12 $\pm$ 0.23	-2.825	0.005
Lp-a (mmol/L)	186.74 $\pm$ 152.61	140.50 $\pm$ 120.15	-1.405	0.174
apoE (mmol/L)	41.53 $\pm$ 15.95	35.00 $\pm$ 7.24	-1.614	0.109

Table 2. General information comparison between CHD group and control group

Index	Group	CHD group (n=155)		Control group(n=102)		χ^2 value	P value
		Cases	Rate (%)	Cases	Rate (%)		
Sex	Male	113	72.9	44	43.1	22.930	<0.001
	Female	42	27.1	58	56.9		
Age	≤50	20	12.9	17	16.7	3.581	0.310
	50~60	52	33.5	42	41.2		
	60~70	53	34.2	25	24.5		
	>70	30	19.4	18	17.6		
	Slim	0	0	3	2.9		
BMI	Normal	75	48.4	35	34.3	12.762	0.005
	Overweight	72	46.5	50	49.0		
	Obesity	8	5.2	14	13.7		
Smoking	Yes	84	54.8	27	26.5	20.134	<0.001
	No	70	45.2	75	73.5		

Table 3. HDL comparisons between control and CHD groups (mg/dl)

Index	Control group(n=102)	CHDgroup(n=155)	T value	P value
Large	22.65±8.38	10.86±4.48	13.037	<0.001
Intermediate	18.50±5.04	20.17±5.73	2.393	0.017
Small	6.17±3.36	10.23±4.03	8.434	<0.001
Total	47.30±10.02	41.28±10.42	4.600	<0.001

Changes of HDL subtypes in patients with HDL decrease in CHD group

Comparisons of HDL < 1.04 mmol/L (40 mg/dl) and HDL subtypes in CHD group: various HDL subtypes decreased, but HDL_L reduced even more significantly (Table 4, 5).

Correlation between HDL subtypes and apoA-I

Large HDL, intermediate HDL, small HDL and total HDL had good correlation with apoA-I, of which, the large HDL had a correlation maximum with apoA-I (Table 6).

Fig. 1. HDL particle subtype map analysis in CHD group.

Correlation between HDL and HDL subtypes

The correlation analysis between HDL and HDL subtypes showed that all subtypes had obvious relevance with HDL, but correlation between HDL_M ($r = 0.911$, $P < 0.001$) and HDL was larger than that between HDL_M ($r = 0.695$, $P < 0.001$) and HDL_S ($r = 0.516$, $P < 0.001$; Table 7; Fig. 3-5).

Fig. 2. HDL particle subtype map analysis in control group. Note: HDL was divided into 10 sub-components, 1-3 were HDL_L, 4-7 were HDL_M, 8-10 were HDL_S. The HDL_L in CHD group was significantly lower, while HDL_L, HDL_M and HDL_S significantly increased.

Table 4. HDL subtype comparison between CHD group and HDL < 1.04 mmol/L

Index	CHD group(n=98)	CHD di group(n=57)	T value	P value
Large HDL	10.86±4.48	8.21±2.56	5.620	<0.001
Intermediate HDL	20.17±5.73	16.13±3.43	6.581	<0.001
Small HDL	10.23±4.03	8.28±3.26	3.858	<0.001
Total HDL	41.28±10.42	32.63±5.23	8.310	<0.001

Table 5. Reduced rate comparisons among subtypes in HDL reduced group

Index	CHD group(n=98)	CHD di group(n=57)	Reduced rate	P value
Large HDL	10.86±4.48	8.21±2.56	24.5%	<0.001
Intermediate HDL	20.17±5.73	16.13±3.43	20%	<0.001
Small HDL	10.23±4.03	8.28±3.26	19.1%	<0.001
Total HDL	41.28±10.42	32.63±5.23	20.1%	<0.001

Table 6. Correlation analysis of different HDL subtypes of CHD and apoA

Index	Correlation coefficient (r)	P value
apoA*Large HDL	0.725	<0.001
apoA*Intermediate HDL	0.432	<0.001
apoA*small HDL	0.544	<0.001
apoA*Total HDL	0.642	<0.001

Table 7. Correlation analysis between total HDL and subtype concentration in CHD group (mg/dl)

Index	Correlation coefficient (r)	P value
Total HDL*Large HDL	0.695	<0.001
Total HDL*Intermediate HDL	0.911	<0.001
Total HDL*small HDL	0.516	<0.001

Discussion

Coronary AS was an important pathophysiological mechanism of CHD development. The oxidized aggregation of LDL lead to the occurrence of AS, and its subtypes were small and dense. sd-LDL was closely related with CHD [8-10].

According to the particle size, LDL can be divided into two types, LDL with particles larger than $265\text{\AA}$ was called big and light LDL (LDL_L), while LDL with particles smaller than $265\text{\AA}$ was called small and dense LDL (sd-LDL). Studies have demonstrated [11] that the sd-LDL concentrations were positively correlated with the incidence of CHD. After collecting clinical specimens, this study firstly detected LDL subtypes by Lipoprint lipoprotein analyzer, then selected LDL specimens with particles larger than $265\text{\AA}$ for further HDL subtype testing, this step avoided the influence of sd-LDL to the experimental results.

Because of its anti-atherosclerotic effect such as reverse cholesterol transport (RCT), anti-oxidation, stabilizing already formed plaques, inhibiting the formed plaque rupture, HDL had extensively studied [4, 5, 12]. Large number of epidemiological studies had confirmed that HDL-C levels were negatively correlated with the incidence of CHD. Prospective cardiovascular munster (PROCAM) [13] study found that the incidence of CHD for patients with HDL-C $> 0.9\text{mmol/L}$ (35 mg/dl) in the 6-year follow-up reduced by about 70% compared with patients with HDL-C less than 0.9 mmol/L (35 mg/dl). Gordon et al. summarized four large prospective epidemiological studies including US Framingham Heart Study (FHS), lipid research clinics prevalence mortality follow-up study (LRCF), coronary primary prevention trial (CPPT) and multiple risk factor intervention trial (MRFIT) [14], and proposed that the occurrence risk of CHD for male decreased by 2%, cardiovascular disease mortality decreased by 3.7% when HDL-C increased by 1mg/dl (0.026 mmol/L), while the occurrence risk of CHD for female reduced by 3%, and cardiovascular disease mortality decreased by 4.7%, and both were independent of other risk factors including LDL. "China Adult Dyslipidemia Prevention Guide" recommended that HDL-C less than 1.04 mmol/L (40 mg/dl) was reduced; greater than or equal to 1.04 mmol/L (40 mg/dl) was appropriate level; greater than 1.55 mmol/L (60 mg/dl) was desired level. US NCEP-ATPIII stressed that HDL-C less than 1.04mmol/L (40mg/dl) was reduced, low HDL-C was

Fig. 3. Correlation scatterplots between HDL_L and HDL in CHD group.

Fig. 4. Correlation scatterplots between HDL_M and HDL in CHD group.

Fig. 5. Correlation scatterplots between HDL_S and HDL in CHD group.

an important risk factor for CHD; greater than or equal to 1.30 mmol/L (50 mg/dl) was desired level; HDL-C greater than or equal to 1.55 mmol/L (60 mg/dl) had a protective role in the prevention of AS. Thus, HDL has long been known as the "anti-atherosclerotic factor" or "cardiovascular protection factor" [15]. However, HDL's role was constantly refreshed [16], the incidence of CHD for patients with HDL gene defect disease did not increase. The ILLUMINATE, RADIANCE, ILLUSTRATE and other studies about cholesterol ester transfer protein, (CETP) inhibitor torcetrapib have found that the use of the drug torcetrapib allowed HDL-C levels significantly increased, but failed to suppress the occurrence and development of atherosclerotic plaques. HPS2 study in 2014 also suggested that only use of niacin to raise HDL did not play the expected protected role from cardiovascular. Clinical work found that although lipidaemia of patients with CHD was standard, and HDL-C levels were normal or even higher, there was still significant vascular residual risk [6, 17, 18]. Therefore, HDL of patients with CHD was not only had amount problems, but also quality problems. Improving HDL function may be more important than raising its level.

HDL volume was the smallest in all lipoproteins. Compared with other lipoproteins, the protein content was the largest, the major apolipoproteins included apoA-I (70%), apoA-II (20%) and a small amount of apoC and apoE, the main phospholipid was liposomes. Studies have shown that apoA-I was closely related with HDL functions, and may be associated with changes of HDL subtypes [13]. HDL synthesis started from liver cells or small intestinal epithelial cells. The adenosine triphosphate binding cassette transporter A1 (ABCA1) located in the basement membrane of the cell transported the phospholipids and cholesterol in the transporter cells apoAI outside the cell and formed pre-mature β -HDL, which can be taken as other sources of cholesterol receptor to accept cholesterol and other ingredients to form mature HDL. The liver and small intestine secreted apoAI, combined with cholesterol and phospholipids outflowing from the liver mediated by ABCA1. The apolipoprotein by LPL hydrolysis from chylomicrons (CMs) and very low density lipoprotein (VLDL) dissociation also combined with apoAI, formed newborn pre-mature β -HDL particles. The premature β -HDL can continuously receive the outflowing cholesterol and phospholipid mediated by ABCA1 in extrahepatic tissues, and formed free cholesterol-rich HDL particles. By the role of plasma cholesterol acyltransferase (LCAT), free cholesterol (FC) esterification transformed cholesterol ester (CE) and into the core part of HDL to form mature α -HDL, HDL particles gradually became larger during maturation.

Human serum HDL had heterogeneity in size, shape, composition and surface charge. On the basis of the content of HDL surface charge, human serum HDL can be divided into α , pre- α , pre- β and γ migration after agarose gel electrophoresis. Of which, the pre-mature β -HDL were disc-shaped particles under the electron microscope, which were nascent HDL particles; α -migration HDL were spherical particles under the electron microscope, which were mature HDL particles. By ultracentrifugation, chemical precipitation, electrophoresis and chromatographic methods [19-21], according to the particle density, α -HDL can be subdivided into two main components including HDL2 (large and sparse HDL, $1.063 < d < 1.125$ g/ml) and HDL3 (small and dense HDL, $1.125 < d < 1.210$ g/ml). The EPIC-Norfolk study [22] found that the average diameter decrease of HDL was related with the risk increases of CHD. Kocaman [23] found that cardiovascular protection function of HDL2 was stronger compared with HDL3, HDL2 decline in patients with was more evident.

This paper divided HDL into 10 subtypes (HDL1-HDL 10) using Lipoprint HDL lipoprotein analyzer, 1-3 types were large particle types (HDL_L), 4-7 types were intermediate particle types (HDL_M), 8-10 types were small particle types (HDL_S). This typing method was different from the traditional HDL2, HDL3 typing methods.

This study found that the HDL_L levels in patients with CHD decreased, while the HDL_M and HDL_S content increased. In addition, The study about CHD patients with HDL < 1.04 mmol/L found that various subtypes of HDL significantly decreased, and the HDL_L had the most significant decrease. The above two points suggested that serum HDL particles of CHD patients became smaller, there may be mature obstacles.

The apoAI was the most abundant lipoprotein in HDL. In the correlation analysis about apoAI, it was found that HDL_L was associated with apoAI at the most compared with HDL_M and HDL_S, therefore, it was concluded that the cardiovascular protective effects of HDL_L may be related with apoAI content.

However, the correlation analysis for HDL and HDL subtypes showed that the greatest correlated subtypes with HDL was HDL_M not HDL_L, which may be caused due to a larger proportion in HDL.

In summary, the results of this study showed that the simple use of HDL level to predict the risk of CHD was one-sided. The HDL_L in serum of CHD patients significantly lowered, HDL had maturation disorders. HDL_L may be a protective factor for CHD, whose decrease was closely related to the risk increase of heart diseases. The cardiovascular protection function of HDL_L may be related with apoAI content.

Disclosure Statement

All authors have no conflict of interest regarding this paper.

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