**Original Article**

**Correlation between high density lipoprotein and monocyte subpopulations among stable coronary atherosclerotic heart disease patients**

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**Abstract:** High density lipoprotein (HDL) is a structurally and functionally heterogeneous molecular particle whose function is unclear in atherosclerosis at present. Studies show that small HDL functional imbalance may exist in Coronary Atherosclerotic Heart Disease (CAD) patients. Monocyte is considered to play an important role in atherosclerosis, in accordance with the expression of superficial CD14 and CD16, it can be divided into three subpopulations. The purpose of this study was to explore the relation between HDL and monocyte subpopulations among CAD patients. We report 90 cases of stable CAD patients and define the monocyte subpopulations as classical monocyte (CD14++CD16-; CM), intermediate monocyte (CD14+CD16+; IM), and non-classical monocyte (CD14+CD16++; NCM); HDL group is measured by polyacrylamide gel electrophoresis. The results indicated that the small HDL in blood serum has a correlation with proinflammatory NCM in circulation but a negative correction with CM and no relationship with diabetes, saccharify hemoglobin, hypertension, smoking history and taking dose of statins drugs and severity of disease. In conclusion, this study primarily confirms that micromolecule HDL level correlates with the increase of non-classical monocyte subpopulations and decrease of classical monocyte quantity. Thus demonstrates the proinflammatory correlation between micromolecule HDL and internal immunity in the development of stable atherosclerosis.

**Keywords:** High density lipoprotein, atherosclerosis, monocyte subpopulations, inflammation

**Introduction**

Although the cardiovascular drugs have been quite developed, cardiovascular disease is still the main cause of death of elderly patients in the world. Most clinic and epidemiology studies demonstrate the closely relationship between high density lipoprotein level and cardiovascular disease, so how to improve uniformity of HDL level becomes the main concern in treatment study [1]. However different from the anticipation, the risk of getting coronary heart disease can not be decreased by improving HDL level through drugs, such as torcetrapib, although it can improve the patients’ HDL level as cholesteryl ester transfer protein (CETP) depressor, a lot of studies show that the prevalence and mortality increase instead [2, 3]. Some researchers suggest that the function of HDL may have been destroyed in the pathological environment, and the key of study and treatment lies in the guarantee of normal function. As a lipoprotein granule equipped with different structure, metabolic function and characteristic of anti-atherosclerosis, small HDL in healthy people has been testified to affect the development of atherosclerosis through increasing cholesterol depletion, anti-oxidation and anti-inflammation and so on. But the function becomes abnormal when atherosclerosis dyslipidemia exists [4-6]. Clinical study reported that there was a correlation between small HDL and the attack and severity of atherosclerosis, while macromolecular HDL has a negative correlation with the attack and severity of CAD. There exists inflammation reaction in the development of atherosclerosis, monocyte plays an important role and can be divided into three...
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subpopulations: classical monocyte (CD14++CD16-; CM), intermediate monocyte (CD14+CD16+; IM), and non-classical monocyte (CD14+CD16++; NCM), the last two kinds are both pro-inflammatory cells. The proportion of pro-inflammatory cells has a correlation with the attack of CAD, and a correlation with intima-media membrane thickness and plaque stability; General cholesterol, LDL cholesterol and triglyceride have a correlation with pro-inflammatory subpopulations NCM, while HDL cholesterol shows to have negative correlation. A study in 900 cases of CAD patients shows that IM has a predicting function to the cardiovascular diseases. The purpose of this study is to research the correlation of different HDL components between CAD patients and distribution of monocyte subpopulations so that we could learn a lesson in the later clinical diagnosis and study.

Materials and methods

Case and study design

We study the cases of stable CAD patients under long-term conserving treatment in our hospital from September, 2009 to August, 2010. Cases standard: patients are over 18 years old and have stable CAD (diagnosed by selective coronary angiography), excluding standard: patients with acute coronary syndrome (ACS) combined with ST segment elevated myocardial infarction (STEMI), non-ST-segment elevated myocardial infarction (NSTEMI) or unstable angina, operated with percutaneous coronary intervention (PCI) and combined with heart failure, cancer and chronic inflammation with liver and kidney and so on. All the cases study of patients are informed consent by the patients themselves and their relatives.
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Blood sample

Blood sample should be acquired from vein in elbow before selective coronary angiography, the first 3 ml blood should be abandoned. Blood should be drawn from EDTA test-tube (Greiner Bio-One) and inspected by flow cytometry, and at the same time put into 3.8% sodium citrate test-tube, serum separation test-tube and EDTA test-tube (Greiner Bio-One), afterwards quickly centrifuged for 15 min under 4°C and 3000 r/min, and stored under -80°C for later analysis.

Laboratory test

Granulocyte colony-stimulating factor (G-CSF), macrophage colony-stimulating factor (M-CSF) and interleukin-6 (IL-6) should be tested through special enzyme-linked immuno-sorbent assay (ELISA; R&D Systems, Minneapolis, MN, USA); interleukin-10 (IL-10) and granulocyte-macrophage colony-stimulating factor (GM-CSF) should be under specific multiple test (Luminex Assay, R&D Systems, catalog number FCST03).

Flow cytometry

Leucocyte and monocyte in blood should be analyzed by FACS Canto II and FACS Diva software (both Becton Dickinson) subpopulations, Figure 1 shows methods of dyeing and gating, 100 μL EDTA anti-coagulant blood should be dyed by monoclonal antibody (mAb) with saturation concentration: corresponding homotype comparison should be made between monoclonal antibody (Beckton, catalog number 345809) marked by CD45 dinoflagellates chlo-

Figure 2. Correlation between small HDL level and monocyte subpopulations.
Correlation between HDL and monocyte subpopulations

Table 1. Correlation between General HDL or Micromolecule HDL or Intermediate HDL or Macromolecule HDL subgroup and the Classical monocyte or Intermediate monocyte or Non-classical monocyte subgroup

<table>
<thead>
<tr>
<th>Monocyte subgroup</th>
<th>Classical monocyte CD14++CD16-</th>
<th>Intermediate monocyte CD14++CD16+</th>
<th>Non-classical monocyte CD14+CD16++</th>
</tr>
</thead>
<tbody>
<tr>
<td>General HDL</td>
<td>-0.08 0.45</td>
<td>-0.06 0.60</td>
<td>0.12 0.25</td>
</tr>
<tr>
<td>Micromolecule HDL</td>
<td>-0.33 <strong>0.001</strong></td>
<td>0.14 0.20</td>
<td><strong>0.30 0.004</strong></td>
</tr>
<tr>
<td>Intermediate HDL</td>
<td>-0.05 0.66</td>
<td>-0.32 0.76</td>
<td>0.07 0.50</td>
</tr>
<tr>
<td>Macromolecule HDL</td>
<td>0.06 <strong>0.055</strong></td>
<td>-0.12 0.26</td>
<td>-0.01 0.96</td>
</tr>
</tbody>
</table>

Bold-type and red letters represent distinct correlation. The P value less than 0.05 represents the positive or negative correlation between the HDL subgroup and the Monocyte subgroup.

acrylamide gel electrophoresis divide HDL into 10 subcomponents, subcomponents 1-3 represent big HDL granule, subcomponents 4-7 represent intermediate HDL subtype and subcomponents 8-10 represent small HDL granule.

Data analysis

All the data statistics and analysis are under the assistance of SPSS 20.0 software, categorical variables are compared through the accurate test of \( \chi^2 \) or Fisher and represented as count or percentage; Sequence variables represent average ± standard deviation; Parameter data is compared by ANOVA. Deflection data is compared by ANOVA after logarithmic conversion; Correlation is calculated by Pearson correlation coefficient; 3 groups of unicellular subpopulations are brought into linear regression analysis model, and at the same time when clinical characteristic, stain drugs treatment or lipid parameter has correlation with unicellular subpopulations or small HDL level (P<0.2), they are also brought into the model; The difference has statistics meaning when P<0.05.

Results

Patient information

Ninety cases of angiography confirm stable coronary heart disease patients in being brought to study. Among which the average age is 64.1±10.0 years old, 72 patients are male (80%), 21 patients are smokers (23%), 25 patients (28%) have single obstructive vessel disease, 36 patients (40%) have double coronary vessel disease and 29 patients (32%) have three coronary disease, 31% patients are under statins drugs with high dose, 52% patients are under statins drugs with low dose and 17% patients haven’t accept any statins treatment.

Correlation between monocyte subpopulations and serum small HDL level

Monocyte subpopulations are tested through flow cytometry (Figure 1). The CM average

Lipid test

Testing the general cholesterol, HDL, LDL and triglyceride level with the above frozen serum sample and making quantitative analysis with the component of HDL subgroup through Quantimetrix HDL Lipoprint system according to the instructions. Specific separation and quantitative mechanism is that high resolution poly
Correlation between HDL and monocyte subpopulations

Table 2. Micromolecule HDL and monocyte subgroup correlation multivariable regression model

<table>
<thead>
<tr>
<th>Micromolecule HDL</th>
<th>Classical monocyte CD14++CD16-</th>
<th>Intermediate type monocyte CD14++CD16+</th>
<th>Non-classical monocyte CD14+CD16++</th>
</tr>
</thead>
<tbody>
<tr>
<td>Univariate P Value</td>
<td>β</td>
<td>P Value</td>
<td>Univariate P Value</td>
</tr>
<tr>
<td>Micromolecule HDL</td>
<td>0.001</td>
<td>-0.33</td>
<td>0.006</td>
</tr>
<tr>
<td>Dose of statins</td>
<td>0.21</td>
<td>0.14</td>
<td>0.19</td>
</tr>
<tr>
<td>Dose of serum</td>
<td>0.36</td>
<td>-0.11</td>
<td>0.35</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.21</td>
<td>0.18</td>
<td>0.48</td>
</tr>
<tr>
<td>Age</td>
<td>0.37</td>
<td>0.09</td>
<td>0.50</td>
</tr>
<tr>
<td>LDL</td>
<td>0.02</td>
<td>-0.19</td>
<td>0.56</td>
</tr>
<tr>
<td>BMI</td>
<td>0.84</td>
<td>0.04</td>
<td>0.71</td>
</tr>
<tr>
<td>General cholesterol</td>
<td>0.02</td>
<td>-0.10</td>
<td>0.77</td>
</tr>
<tr>
<td>Smoking history</td>
<td>0.51</td>
<td>-0.03</td>
<td>0.78</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.49</td>
<td>-0.03</td>
<td>0.81</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>0.71</td>
<td>0.06</td>
<td>0.81</td>
</tr>
<tr>
<td>Gender</td>
<td>0.96</td>
<td>0.01</td>
<td>0.99</td>
</tr>
<tr>
<td>General model R2</td>
<td>21%</td>
<td>0.040</td>
<td>24%</td>
</tr>
</tbody>
</table>

Bold-type and red letters represent distinct correlation. The P value less than 0.05 represents the positive or negative correlation.

Figure 3. Correlation between monocyte subpopulations and micromolecule HDL level grade (3 levels).
Correlation between HDL and monocyte subpopulations

Table 3. Correlation between HDL subgroup and lipid parameter

<table>
<thead>
<tr>
<th></th>
<th>Micromolecule HDL</th>
<th>Intermediate type HDL</th>
<th>Macromolecule HDL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>P Value</td>
<td>R</td>
</tr>
<tr>
<td>HDL</td>
<td>0.060</td>
<td>0.56</td>
<td>0.68</td>
</tr>
<tr>
<td>General cholesterol</td>
<td>0.39</td>
<td>&lt;0.0005</td>
<td>0.60</td>
</tr>
<tr>
<td>LDL</td>
<td>0.30</td>
<td>&lt;0.0005</td>
<td>0.43</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.42</td>
<td>&lt;0.0001</td>
<td>0.22</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>0.36</td>
<td>&lt;0.0005</td>
<td>0.02</td>
</tr>
<tr>
<td>Micromolecule HDL</td>
<td>-</td>
<td>0.01</td>
<td>0.91</td>
</tr>
<tr>
<td>Intermediate type HDL</td>
<td>0.01</td>
<td>0.91</td>
<td>-</td>
</tr>
<tr>
<td>Macromolecule HDL</td>
<td>-0.26</td>
<td>0.014</td>
<td>0.63</td>
</tr>
</tbody>
</table>

HDL: high density lipoprotein; LDL: low density lipoprotein; VLDL: ultra-low density lipoprotein; Bold-type letters represent distinct correlation. Bold-type and red letters represent distinct correlation. The P value less than 0.05 represents the positive or negative correlation.

Numerical value is 270.5±142.7 cells/μL (taking up 82.1±6.7% general monocyte), the average NCM numerical value is 39.7±28.9 cells/μL (taking 12.3±5.9% monocyte), IM average is 18.7±15.1 cells/μL (taking 5.6±3.3% general monocyte). There is a negative correlation between micromolecule HDL in serum and CM proportion in peripheral circulation (r=-0.33, P=0.001, Figure 2A), micromolecule HDL in serum correlates with NCM (r=0.30, P=0.004, Figure 2B) and doesn’t correlate with IM (r=0.14, P=0.20, Figure 2C). There is no correlation between intermediate type HDL, macromolecule HDL, general HDL and monocyte subpopulations as expressed in Table 1. Linear regression analysis demonstrates that micromolecule HDL independently correlate with pro-inflammatory NCM and CM in peripheral circulation compared with other lipid parameter, risk factor and statins drugs. Table 2 demonstrates that IM only relates with general cholesterol and LDL. The comparison between the patients’ intermediate tertile (9-12 mg/l) and lowest tertile (2-8 mg/l) shows that CM value is the lowest (79.3±7% vs. 83.7±6% and 83.9 ± 6%; P=0.004, Figure 3A) when the patient’s small HDL level reaches top 1/3 of the maximum value (13-20 mg/dl). In addition, small HDL which reach top 1/3 of the maximum value has highest level of pro-inflammatory NCM big (14.7±7% vs. 10.7±5% and 10.8±5%, P=0.006, Figure 3B), IM doesn’t correlate with small HDL tertile (5.9±3% vs. 5.6±3% vs. 5.3±3%, P=0.54, Figure 3C).

Correlation between 2HDL subpopulations and lipid parameter and cardiovascular risk factors

Small HDL level doesn’t correlate with general HDL level but distinctly correlates with the triglyceride, VLDL, LDL and general cholesterol level (Table 3); There is a negative correlation between the big HDL and the small HDL, VLDL and triglyceride but closely correlate with general cholesterol, intermediate type HDL correlates with macromolecule HDL and LDL, VLDL. What micromolecule HDL differentiates with intermediate type HDL is that the latter doesn’t correlate with triglyceride. And intermediate type HDL level (28.8±7.1 vs. 23.7±5.8 mg/dl, P=0.002) among female patients is obviously higher than that of male patients, while micromolecule HDL level has no gender difference (13-20 mg/dL). The comparison between the patients’ intermediate tertile (9-12 mg/l) and lowest tertile (2-8 mg/l) shows that CM value is the lowest (79.3±7% vs. 83.7±6% and 83.9 ± 6%; P=0.004, Figure 3A) when the patient’s small HDL level reaches top 1/3 of the maximum value (13-20 mg/dl). In addition, small HDL which reach top 1/3 of the maximum value has highest level of pro-inflammatory NCM big (14.7±7% vs. 10.7±5% and 10.8±5%, P=0.006, Figure 3B), IM doesn’t correlate with small HDL tertile (5.9±3% vs. 5.6±3% vs. 5.3±3%, P=0.54, Figure 3C).

Relationship between HDL subgroup and circulating cells colony stimulating factor and inflammation sign

Small HDL level in serum distinctly correlates with blood plasma G-CSF (r=0.22, P=0.05), but doesn’t correlate with GM-CSF (r=0.05, P=0.66) or M-CSF (r=0.09, P=0.37). Intermediate type HDL, macromolecule HDL and general HDL do not correlate with three species of colony stimulating factors. Small HDL level in serum doesn’t correlate with pro-inflammatory reaction mark hsCRP (r=-0.05, P=0.64), IL-6 (r=-0.10, P=0.38) and IL-10 (r=0.06, P=0.62). In addition, there is no correlation respectively between middle-density lipoprotein, big HDL, general HDL and hsCRP, IL-6, sum IL-10. Monocyte subpopulations do not correlate with pro-inflammatory cell factors hsCRP (CM: R=-0.11, P=0.32, IM: R=0.14, P=0.19; NCM:
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R=0.04, P=0.68) or IL-6 (CM: R =0.06, P=0.59; IM: R=0.13, P=0.24; NCM: R=-0.14, P=0.24) or anti-inflammatory mark IL-10 (CM: R=0.01, P=0.94; IM: R=-0.01, P=0.98; NCM: R=0.01, P=0.94).

Discussion

At present, a great many epidemiology and forward-looking studies have demonstrated clearly that there is a negative correlation between HDL level in serum and attack risk of coronary heart disease, the arterial protection function of HDL shown in the outflow of cholesterol, anti-oxidation, anti-inflammatory reaction, cellular protection and vasodilator anti-thrombosis and so on [7-12]. At the same time a good many studies also confirm that small HDL granule have potential function of anti-atherosclerosis, when dyslipidemia occurs, such as with the rise of simple triglyceride level or general cholesterol, increase of small HDL granule and decrease of big HDL granule is capable of changing the metabolic and subpopulations distribution. In this study, 90 patients is confirmed with stable coronary heart disease through angiography, and small HDL level in serum correlates with atheromatous blood fat indicators such as general cholesterol, LDL, VLDL and triglyceride level, and doesn't correlate with general HDL, serum lipoprotein level and statins drugs taking history.

Evidence shows that transform of small HDL level not only occurs to dyslipidemia and obesity patients, but also attacks betide patients with cardiovascular disease [13-16]. 115 patients through coronary radiography shows that macromolecule HDL level obviously increases for those who have coronary disease; 10 years' follow up visit of 1000 patients confirms that micromolecule HDL and macromolecule HDL have predicting function for the development of ischemic heart disease, the function disappears once the small HDL level is adjusted. With the gradual decrease of HDL granule, the relationship with female CAD morbidity becomes closer, and small HDL level increases in acute ischemic shock patients, compared with healthy people; A clinical study of 60 patients shows that small HDL correlates with lager unstably non-calcified plaque confirmed by coronary CT and ultrasonic in vessel; Another study of 102 myocardial infarction patients and 200 patients as control group shows that big HDL and intermediate type HDL has a negative correlation with early acute myocardial infarction, if the small HDL level in young acute myocardial infarction increases, then the small HDL in acute coronary syndrome also increases, while macromolecule HDL level decreases. HDL subgroup correlates with severity of disease [17, 18].

The marks on the monocyte surface CD14 and CD16 have already confirmed the relationship between the heterogeneity of monocyte and atherosclerosis, CD16 positive monocyte increases in the development acute and chronic inflammation and atherosclerosis and activated quickly after inflammatory stimulation [19]. This study preliminarily confirms that patients correlate with proinflammatory monocyte subpopulations distribution in the situation of having stable coronary artery disease and increase of micromolecule HDL level, non-classical monocyte (CD14+CD16++) level increases and classical monocyte (CD14++CD16-) level decreases, and intermediate type monocyte (CD14++CD16+) doesn’t correlate with micromolecule HDL in serum. Study confirms that the correlation between general HDL or LDL and monocyte subpopulations disappear after the adjustment of BMI. The result of this study is independent to traditional age, gender, smoking history, BMI, diabetes and statins drugs taking history. Patients are divided into 3 grade groups according to their small HDL level, the maximum level group shows distinct monocyte subpopulations’ promoting arterial atherosclerosis and pro-inflammatory reaction, the CM proportion decline is accompanied with the increase of NCM proportion. However monocyte subpopulations have no correlation with CRP, IL-6, IL-10 and other inflammation marks. Study reports at present that CD16 positive monocyte have correlation with hsCRP level in jitter angina patients, and other studies also confirm that CD16 positive monocyte have no relationship with hsCRP or IL-6 but have correlation with TNF-α level, therefore dispute still exists [20]. In addition many vessel cells are tested to express colony stimulating factors CSFs and be capable of responding it. CSFs could affect the development of arterial atherosclerosis through adjusting the macrophage phenotype and cholesterol intake [21, 22]. This study shows that blood plasma G-CSFs level

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distinctly correlates with micromolecule HDL. But confined to horizontal analysis, we could only analyze the relationship between HDL subpopulations and monocyte at certain time, and couldn’t judge the functional change of this relationship in the process of arterial atherosclerosis.

In conclusion, this study primarily tests that micromolecule HDL level correlates with the increase of non-classical monocyte subgroup and the decrease of classical monocyte subgroup, and demonstrates the proinflammatory relationship between micromolecule HDL and internal immunity in the development of stable atherosclerosis.

Acknowledgements

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Disclosure of conflict of interest

None.

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