Serum High Density Lipoprotein Subfractions and Other Lipid Parameters in Coronary Artery Diseases

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Conventionally, high density lipoprotein cholesterol (HDL-C) level was measured in prediction of coronary artery disease (CAD). Because HDL particles are continuously remodeled in terms of lipid and apolipoprotein composition, the distribution of cholesterol in HDL subclasses; HDL2-Cholesterol (HDL2-C) and HDL3-Cholesterol (HDL3-C) levels are more predictive values for occurrence of CAD than total HDL-C level. The aim of this study was to investigate the HDL2-C and HDL3-C levels in patients who had undergone coronary angiogram in Yangon General Hospital and to investigate the association between serum HDL2-C level, HDL3-C level and occurrence of CAD. Pre-procedural fasting serum were collected from 132 patients and analyzed for HDL2-C, HDL3-C, total cholesterol (TC), total HDL cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and triglyceride (TG). The TC, HDL-C and TG levels were determined by enzymatic colorimetric method and LDL-C levels were calculated from the above-mentioned lipid parameters. The median serum HDL-C levels were significantly lower in CAD patients (35.73 mg/dl) than patients with normal angiogram (40.95 mg/dl). The median serum HDL2-C levels were 11.93 mg/dl in significant CAD patients and 18.39 mg/dl in patients with normal angiogram. The median serum HDL3-C levels were 22.54 mg/dl in significant CAD patients and 22.93 mg/dl in patients with normal angiogram. Patients with significant CAD had significantly lower median serum HDL2-C level than patients with normal angiogram ($p<0.01$). There was no significant difference in median HDL3-C level between patients with significant CAD and patients with normal angiogram ($p>0.05$). There was significant association between CAD and decreased level of HDL2-C ($p<0.05$). The fall in serum HDL 2-C level is more likely to be predictive of CAD risk than HDL3-C level. Therefore, low serum HDL2-C level is associated with increased CAD risk.

Keywords: HDL2 cholesterol, HDL subfractions, Coronary artery disease

INTRODUCTION

Coronary artery disease (CAD) is a life-threatening disease associated with severe morbidity and mortality.[1] According to the Annual Hospitals Statistics Report 2012 from Ministry of Health, CVDs account for 5.2 percent of overall morbidity and 17.1 percent of overall mortality in Myanmar. For the development of CAD, dyslipidemia such as increased total cholesterol and LDL cholesterol (LDL-C) and decreased high-density lipoprotein cholesterol (HDL-C) concentration play as an important factor.[2, 3] Many epidemiologists have shown there is an inverse and independent relationship between HDL-C and risk of cardiovascular disease.[4] When the concentration of HDL-C is increased decreased by about 2% to 3%.[5] In Myanmar, the inverse relationship

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DOI: https://doi.org/10.34299/mhsrj.00982

between HDL-C and CAD have been shown in Sunn (2003).[6]

However, the elevation of HDL-C alone did not retard atherosclerosis progression or reduce the incidence of cardiovascular events.[7] In contrast, some therapies by improving HDL function such as enhancing reverse cholesterol transport or changing HDL particles distribution could show greater benefit.[8, 9] The reason for the disparity of these studies might be explained by the fact that HDL consists of a heterogenous group of particles differing not only in size and density, but also by chemical composition and physiological function.[10, 11] In fact, the exact role of HDL particles of different sizes in CAD pathogenesis has not been fully evaluated.

Meta-analysis done by Superko, *et al*[10] and Pirillo, Norata & Catapano[12] showed the controversial results between CAD risk and HDL subclasses separated by density. A majority of the studies, however, have found HDL2-C to be more predictive of CAD risk than total HDL or HDL3-C.[13, 14] The 29-year follow-up of the Gofman's Livermore cohort showed that HDL2 and HDL3 are independently related to CAD risk.[15] The recent studies of Xu, *et al*[16, 17] showed that the percentage of large HDL subfraction was negatively associated with the severity of CAD and percentage of small HDL subfraction was positively associated with the severity of CAD. In Myanmar, analysis of serum HDL2-C and HDL3-C level of CAD patients have not been verified. Thus, this study is aimed to find out the serum HDL2-C and HDL3-C levels of CAD patients, and the association between the serum HDL2-C, HDL3-C levels and occurrence of CAD.

MATERIALS AND METHODS

A total of 132 patients who would had been undergone cardiac angiogram at Cardiac Medical Unit of Yangon General Hospital during the period of May 2016 and June 2017 participated in the hospital-based comparative study. After getting the written informed consent, the patients were instructed for overnight fasting. After that, their fasting blood sample was taken. The data of the coronary angiogram of the patients was also recorded and the presence and absence of CAD were categorized into two groups in data analysis. The blood pressure, weight and height were taken from patient medical record. The BMI was calculated from patient's weight and height. The body fat percent was calculated by the formula.[18] The presence of risk factors such as diabetes mellitus and hypertension were found out by questionnaires. There were no exclusion criteria in selection of patient. The patients who were currently taking lipid lowering therapy were also included in current study.

The fasting sera were then analyzed for HDL2-C and HDL3-C levels by single precipitation procedure with dextran sulphate, heparin sodium and $MnCl_2$ solution followed by enzymatic colorimetric assay for cholesterol.[19] The serum total cholesterol (TC) and triglyceride (TG) levels were also determined by enzymatic colorimetric method.[20, 21] Serum low-density lipoprotein cholesterol (LDL-C) was determined by using formula modified by McNamara, *et al* (1990).[22]

Data entry and analysis were done by using Statistical Package for Social Sciences, SPSS 21 version. Summary statistics of the range, the mean, the median, the standard deviation, interquartile range and the percentiles were calculated for HDL-C, HDL2-C, HDL3-C, HDL2-C/HDL3-C, TC and TG. Due to the skewness of the data, comparisons of the HDL-C, HDL2-C, HDL3-C, HDL2-C/HDL3-C, TC and TG in CAD and control were done by Mann-Whitney U test. The associations between HDL-C, HDL2-C, HDL3-C, HDL2-C/HDL3-C and CAD were calculated by Pearson Chi-square (χ^2) test. Correlations of HDL-C, HDL2-C, HDL3-C, HDL2-C/HDL3-C, TC and TG were calculated by Spearman correlation coefficient (ρ). The stronger significant level was set at $P<0.05$.

RESULTS

The baseline characteristics of the 132 participants (72 CAD patients and 60 control patients) are shown in Table 1. The BMI of the CAD patients were significantly higher than patients without CAD (26.36 kg/m^2 *vs* 23.34 kg/m^2, $p<0.001$).

Table 1. Baseline demographic of the patients with CAD and controls

Characteristics	CAD n=72	Without CAD n=60	p value
Age (Years)	57.5(16)	55(17)	0.149
BMI (kg/m^2)	26.36(5.73)	23.34(6.87)	<0.001
Body fat (%)	29.96(11.7)	29.08(13.12)	0.676
Systolic blood pressure (mmHg)	113.5(26.0)	117(26.5)	0.62
Diastolic blood pressure (mmHg)	72(10.75)	70(18)	0.177
*Results are shown in median (IQR) and calculated by Mann-Whitney U test			
Sex			
Male	51(70.8)	23(38.3)	<0.001
Female	21(29.2)	37(61.7)	
Hypertension n(%)	39(54.2)	19(31.7)	0.01
Diabetes n(%)	23(31.9)	3(5)	<0.001
History of smoking n(%)	25(34.7)	18(30)	0.564
*Results are shown in number (percentage) and calculated by Pearson Chi-square test			

In CAD group, male sex occupied 70.8% which was much greater than 38.3% in control group. The occurrence of CAD was significantly associated with male sex ($p<0.001$). The risk factors such as hypertension and diabetes mellitus were also significantly associated with CAD. There was no significant association between history of smoking and occurrence of CAD.

The HDL-C, HDL2-C and HDL2-C/ HDL3-C were significantly higher in control patients than in CAD patients (Table 2). HDL3-C tended to be slightly higher in control patients than CAD patients, though it did not reach a significant level. TC and LDL-C were also significantly higher in control patients than CAD patients. The association between HDL-C, HDL2-C and

Table 2. Fasting lipids parameters in patients with CAD and controls

Parameters	CAD n=72	Without CAD n=60	*Z* value	p value
HDL-C (mg/dl)	35.73 (10.1)	40.95 (17.48)	-3.115	0.002
HDL2-C (mg/dl)	11.93 (13.99)	18.39 (18.03)	-2.792	0.005
HDL3-C (mg/dl)	22.54 (9.03)	22.93 (10.56)	-0.747	0.455
HDL2-C/HDL3-C ratio	0.49 (0.95)	0.76 (0.89)	-2.098	0.036
TC (mg/dl)	141.3 (49.08)	162.54 (64.24)	-2.868	0.004
LDL-C (mg/dl)	72.3 (52.08)	91.39 (48.12)	-2.774	0.006
TC/HDL ratio	3.9 (1.46)	3.58 (1.59)	-0.407	0.684
TG (mg/dl)	129.67 (95.52)	136.09 (106.86)	-1.156	0.248

Results are shown in median (IQR) and calculated by Mann-Whitney U test

Table 3. Association of different HDL-C and CAD

	CAD n (%)	Without CAD n (%)	p value
HDL-C (>37.23 mg/dl)	29 (44.6)	36 (55.4)	0.024
HDL2-C (>14.49 mg/dl)	30 (45.5)	36 (54.5)	0.036
HDL3-C (>22.66 mg/dl)	34 (51.5)	32 (48.5)	0.484

Cut-off points for HDL-C, HDL2-C and HDL3-C were taken from the median value of their respective parameters. The associations were calculated by Pearson chi square test.

HDL3-C levels and occurrence of CAD in this study is shown in Table 3. The cut-off points were defined by median value cholesterol levels in all subjects. The decrease in HDL-C and HDL2-C was significantly associated with occurrence of CAD ($p<0.05$). The decrease in HDL3-C was not associated with occurrence of CAD ($p>0.05$).

The HDL-C, HDL2-C and HDL3-C were all strongly and positively correlated with serum TC (Spearman's correlation coefficient 0.437, 0.242 and 0.211, respectively). HDL2-C and HDL3-C were also strongly and positively correlated with HDL-C (Spearman's correlation coefficient 0.796

Table 4. Correlation matrix for serum lipid measurements

	Serum TC	Serum HDL-C	Serum HDL3-C	Serum HDL2-C	Serum TG	Serum LDL-C
Serum TC	1.000					
Serum HDL-C	0.437**	1.000				
Serum HDL3-C	0.211*	0.197*	1.000			
Serum HDL2-C	0.242**	0.796**	-0.361**	1.000		
Serum TG	0.373**	-0.023	0.058	-0.076	1.000	
Serum LDL-C	0.879**	0.219*	0.109	0.069	0.066	1.000

Values inside each box represented Spearman's rho, the pairwise correlation coefficient

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

and 0.197, respectively). There were no significant correlations between TG, LDL, HDL2-C and HDL3-C (Table 4).

DISCUSSION

Understanding of the HDL subfractions, rather than HDL-C alone, provides more insight on enhancing reverse cholesterol transport and redistribution of cholesterol to other lipoprotein particles. This may have greater benefit for effective lipid therapies in prevention and treatment of cardiovascular disease.

Prior work on the association of HDL2 and HDL3 with CVD has yielded conflicting results. In a meta-analysis, it was reported that 45% of 37 total case-control studies found a significant decrease in both HDL2 and HDL3 levels in CVD cases, 26% found a significant decrease in HDL2 only, 11% found a significant decrease in HDL3 only, and 17% found no significant decrease in either HDL subfractions.[10]

In the present study, the fasting serum lipids parameters (TC, HDL-C, HDL2-C, HDL3-C, TG, VLDL and LDL) were investigated in voluntarily participated 132 patients, 72 patients with CAD and 60 patients without CAD. Coronary artery disease diagnosed by angiography may be more specific than by ECG. Therefore, the patients who had undergone coronary angiogram for various cardiac problems in cardiac medical unit in Yangon General Hospital were recruited in this study.

The TC and LDL-C were significantly increased in patients without significant CAD in this study. This result may be mainly due to involvement of patients with lipid lowering therapy in significant CAD group. Most patients resulting significant CAD were taking lipid lowering therapy more than 8 weeks. This may affect the TC and LDL-C levels of the patients with significant CAD.

In the present study, as agreed in all other studies, HDL-C was significantly higher in patients without CAD than patients with CAD (p=0.002) and the decreased in HDL-C was significantly associated with the occurrence of CAD (p=0.024). After fractionation, it was found that HDL2-C was significantly higher in patients without CAD than in patients with CAD (p=0.005) and there was also a significant association between HDL2-C and CAD (p=0.036).

The HDL3-C was also increased in control patients than CAD patients though it did not attain a significant level in both comparison and association. According to these results, the decrease in both serum HDL-C and HDL2-C may contribute to predict CAD risk.

In the present study, the HDL3-C level was higher than the HDL2-C level in total study population. This was consistent with the findings of Smuts and coworkers[23] who studied on Caucasian CAD patients. Kim and colleagues[24] also reported similar results who studied on patients with carotid artery disease (CAAD) in Korea.

However, in the Japanese subjects studied by Hirano, *et al*,[19] the HDL2-C level was higher than HDL3-C level. Although the reason for this discrepancy is unclear, it appears to be affected by genetic factors. For example, Hepatic Lipase (HL) activity and HL gene promoter polymorphism are the important

determinants of HDL2-C. One allelic variation in the HL gene associated with higher HDL2-C was more frequent in Japanese population. In addition, cholesterol ester transfer protein (CETP) activity and its gene polymorphism were associated with the variance in HDL3-C. Such factors may influence whether HDL2-C or HDL3-C predominate in certain populations.[25] The present study is the first study in Myanmar for studying HDL-C subfractions and it is still needed to verify the dominant pattern of HDL2-C and HDL3-C fractions in a large-scale study.

According to Table 3, HDL-C and HDL2-C were shown to have inverse associations with CAD. Salonen, *et al* [26] reported that both HDL-C and HDL2-C levels had inverse associations with the risk of acute myocardial infarction and could be protective factors in ischemic heart disease whereas the role of HDL3-C remained equivocal. In the present study, there was no significant change in HDL3-C between CAD and control group, and therefore, it could not be stated that HDL3-C could be cardioprotective marker.

The HDL2-C was inversely correlated with ApoB and sdLDL which was strongly associated with atherosclerosis. However, it was also reported that HDL3-C level was positively correlated with LDL-C and apoB levels, which are recognized risk factors for atherosclerosis.[25] The correlations of HDL3-C to atherosclerosis were varied in different studies. Some studies stated that HDL3-C was risk factor for atherosclerosis, some stated that it was cardioprotective marker and some reported that there was no significant correlation between HDL3-C and atherosclerosis.

Many studies have established that serum TG level was negatively correlated with HDL2-C. Serum TG level is reflected by the presence of TG rich ApoB containing particles in serum. Through the actions CETP, transfer of TG to HDL particles leads to the generation of TG-rich HDL2 particles, which are more susceptible to be modified by hepatic lipase. Hepatic lipase then converts HDL2 particles to HDL3 particles, which may impair its cardioprotective function.[27]

In the present study, the serum TG level was tending to be increased in patients without CAD than patients with significant CAD (129.67 mg/dl *vs* 136.09 mg/dl) but did not attained the significant level. There was also no significant correlation between serum HDL2-C, HDL3-C, LDL-C and TG (Table 4). This may be due to effects of statin therapy which can greatly decrease the LDL-C and TG level of the patients.[28]

The relationships of HDL subclasses with the cardioprotective mechanisms of HDL, including reverse cholesterol transport, and anti-inflammatory, anti-oxidative effects, and anti-thrombotic effects are not well understood. The antiatherogenic properties of HDL have been primarily ascribed to reverse cholesterol transport. The promotion of reverse cholesterol transport depends partly on the cholesterol efflux capacity of HDL, i.e. their acceptance of cholesterol from macrophages. Khera, *et al* [29] reported that HDL efflux capacity was inversely correlated with carotid intima-media thickness, and that this association remained statistically significant when adjusted for HDL cholesterol levels.

The association of low HDL2b to cardiovascular disease is thought to be primarily due to compromised ABCG1-mediated efflux of cellular cholesterol to larger HDL.[30] There is also ABCA1-mediated efflux of cellular cholesterol to preβ HDL.[31] The functions of ABCA1 and ABCG1 may be coordinated, with ABCA1 initially providing phospholipid to lipid-poor pre-β HDL, followed by ABCG1 providing additional phospholipid and cholesterol enrichment. In familial hypercholesterolemia, the capacity of large HDL2 to mediate free cholesterol efflux via both scavenger receptor-B1 and ABCG1-dependent pathways is reportedly reduced, and carotid intima-media thickness was found to correlate inversely with both scavenger receptor-B1-dependent and

ABCG1-dependent HDL2 efflux capacities.[32] Plasma HDL2 concentrations may also reflect the catabolism and clearance of atherogenic triglyceride-rich lipoprotein remnants.[33] These previous findings also support the inverse relationship of HDL2-C with the occurrence of CAD in this study.

Different mechanisms may endow HDL3 with its cardioprotective properties. HDL3 particles are less prone to oxidation than HDL2, possibly due to paraoxonase 1 activity, which is greater for HDL3 than HDL2.[34] Oxidized LDLs are considered atherogenic and small, dense HDL3 particles appear to inhibit LDL oxidation to a greater extent than HDL2.[35] Electrophoresis appears to provide greater resolutions of HDL subclasses, which may become increasingly important in identifying individuals at risk and defining therapies for preventing or reversing atherosclerosis.[36] The one-step precipitation method used in the present study can provide the serum HDL2-C level which was shown to correlate significantly with HDL2b separated by electrophoresis method.[13] So, the HDL2-C level can also be applicable to provide greater insight in prevention and treatment of CAD therefore the electrophoresis method can be recommended for prediction on risk of CAD.

Recommendation

Further studies are required to evaluate cut-off points for HDL-C, HDL2-C and HDL3-C among Myanmar people. More insightful investigations are also required to determine the effects of statin therapy on HDL-C, HDL2-C and HDL3-C. To understand the underlying mechanism of HDL particles in occurrence of CAD, the Apo-A proteins level, LCAT activity, hepatic lipase activity and cholesterol ester transfer protein (CETP) activity should be measured along with HDL2-C and HDL3-C levels. Relationship between HDL2-C, HDL3-C and severity of CAD, according to clinical scoring system, also needs to be investigated.

In conclusion, the present study examined the serum lipid profile including subfraction analysis of HDL in the patients who would have been coronary angiogram in cardiac medical unit, Yangon General Hospital. The serum HDL-C and HDL2-C levels in CAD patients were significantly lower than those found in patients without CAD. HDL-C and HDL2-C levels were also strongly and negatively associated with occurrence of CAD. According to these results, both HDL-C and HDL2-C were significantly associated with the occurrence of CAD. However, it was not seen in this study that HDL2-C was superior to HDL-C.

Competing interests

The authors declare that they have no competing interests.

ACKNOWLEDGEMENT

We would like to express our heartfelt thanks and gratitude to Professor Nwe Nwe, all the doctors and nurses from Cardiac Medical Unit, YGH for their helpful guidance and assistance during the research.

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