HDL functions and their interaction in patients of Acute Coronary Syndrome: a case control study

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Research

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Abstract

Aim: Recent studies emphasize the importance of HDL function over HDL cholesterol measurement, as an important risk for cardiovascular diseases (CVD). We compared the HDL function of patients with Acute Coronary Syndrome (ACS) and healthy controls.

Methods: We measured cholesterol efflux capacity of HDL using THP-1 macrophages labelled with fluorescently tagged (BODIPY) cholesterol. Paraoxonase and arylesterase activity of PON1 enzyme were assessed by spectrophotometric methods.

Results: We recruited 151 ACS patients and 110 controls. The HDL function of all patients during acute phase and at six month follow-up was measured. The mean age of the patients and controls was 51.7 and 43.6 years respectively. The mean HDL cholesterol/apolipoprotein A-I levels of patients during acute phase, follow-up and of controls were 40.2 mg/dl/ 112.5 mg/dl, 38.3 mg/dl/ 127.2 and 45.4 mg/dl/ 142.1 mg/dl respectively. The cholesterol efflux capacity of HDL was positively correlated with apolipoprotein A-I levels during acute phase ($r = 0.19$, $p = 0.019$), follow-up ($r = 0.26$, $p = 0.007$) and of controls ($r = 0.3$, $p = 0.0012$) but not with HDL-C levels. Higher levels of CEC, PON 1 activity and apolipoprotein A-I were associated with lower odds of development of ACS. We also observed that combined analysis of two HDL functions could improve the predicted probability of ACS development.

Conclusion: ACS is associated with reduced HDL functions which improves at follow-up. The predicted probability of ACS depends upon individual HDL functions and the interactions between them.

Introduction

High density lipoprotein-cholesterol (HDL-C) levels have been associated with atheroprotective effects in epidemiological studies [23]. However, result of multiple clinical trials with HDL-C raising agents (niacin and CETP inhibitors) have shown no cardiovascular benefit with increase in HDL levels [1, 17]. Studies using Mendelian randomisation have also refuted inverse association of HDL-C levels with cardiovascular diseases (CVD) [12, 26].These findings and the knowledge of the complex nature of HDL as a heterogenous group of molecules containing a large number of proteins with several pleiotropic functions has brought the focus on assessing HDL function in addition to HDL-C levels for CVD risk stratification [9, 16].

Cholesterol efflux capacity of HDL allows it to remove excess cholesterol from the macrophages by the process of reverse cholesterol transport and promote its excretion from the body though bile. This property of HDL was found to be associated with lower CVD risk independent of the HDL-C concentration [20] HDL particles also have antioxidative properties and prevent oxidative modification of LDL particles that induce a proinflammatory profile in the vessel wall [4].

Our study aimed to analyze the cholesterol efflux capacity and antioxidative activity (as reflected in Paraoxonase 1(PON1) activity) of HDL in patients of acute coronary syndrome. The study objectives were to compare the HDL functions between healthy controls and patients presenting with acute coronary syndromes, evaluate the changes in the functional parameters after six months of treatment followed by an assessment of the relationship of both CEC and PON1 activity with HDL-cholesterol and Apo A-I. We have also studied the interaction between HDL functions that we analyzed, as predictors of the probability of development of acute coronary syndrome.

Methods

Study participants

We recruited 150 ACS cases and 110 control subjects. Patients presenting with ACS were recruited from the Cardiothoracic Centre at All India Institute of Medical Sciences, New Delhi, India. ACS was defined as ischemic symptoms with ST segment elevation (more than 1 mm ST segment elevation in contiguous limb leads/more than 2 mm ST segment elevation in precordial leads) (STEMI) or with raised cardiac biomarkers (troponin) with or without ST segment changes in ECG (NSTEMI). Patients with inflammatory or autoimmune disorders, on thyroid medication or on statins were excluded. The control subjects included in the study were healthy adults with no clinical cardiovascular disease and not on any lipid-lowering therapy. Control group comprised of both volunteers with no history of cardiovascular diseases and subjects in whom no coronary occlusion was observed after undergoing CT angiography. The study protocol was approved by Institute Ethics Committee of All India Institute of Medical Sciences, New Delhi (Reference Number: IESC/T-380/17.10.2014). Signed informed consent was obtained prior to enrolling in the study. The participants did not receive any stipend for taking part in the study.

Blood sample collection and biochemical parameters

Blood samples were collected at the time of presentation with ACS before administration of statins. The follow-up blood samples were collected at six months while on treatment, which included a statin (atorvastatin) along with other cardioprotective drugs as per treating physician prescription. Samples were centrifuged at 2500xg for 20 min, and after separation of serum, multiple aliquots of serum were immediately stored at minus eighty degree Celsius (-80℃) until further processing. Lipid profile of all participants were done using the AU480 auto analyzer (Beckman Coulter, California, United States). Apolipoprotein A-I (apo A-I), Apolipoprotein B (apo B) and high sensitivity C reactive protein (hs-CRP) levels were measured using an immunoturbidimetric method.

Determination of HDL functions

Cholesterol efflux capacity

Macrophage specific cholesterol efflux was performed according to a previously described protocol with modifications [22]. ApoB depleted serum isolated using 20% polyethylene glycol was used as cholesterol acceptor in the assay. To measure the cholesterol efflux capacity of HDL, THP-1 human monocytes were differentiated into macrophages by the addition of 50 nM phorbol myristate acetate (PMA). Expression of

ATP binding cassette transporter 1 (ABCA1) was analyzed using real-time PCR after differentiation. Differentiated THP-1 macrophages were labelled with BODIPY cholesterol. Labelling was done by incubating the cells in labelling media containing 0.025 mM BODIPY-cholesterol, 0.1 mM unlabeled cholesterol and 10 mM methyl beta cyclodextrin for 1 hour followed by equilibration in Roswell Park Memorial Institute (RPMI) 1640 medium containing 0.2% bovine serum albumin for 15 hrs. Thereafter, cells were incubated with apoB depleted serum for 4 hours. After 4 hrs, media was removed, centrifuged and fluorescence intensity was measured (excitation 482 nm, emission 515 nm). To calculate percent efflux, fluorescence intensity of the cells was also measured after equilibration time by solubilizing the cells in sodium deoxycholate. Cholesterol efflux capacity was calculated as [(fluorescence intensity with acceptor- fluorescence intensity without acceptor)/fluorescence intensity of cells after equilibration time].

Pooled serum from five healthy volunteers was used with every plate to correct for inter-assay variation, and values for cholesterol efflux from patient's serum were normalized to this pooled serum values in subsequent analyses. All samples were run in triplicates. Inter assay coefficient of variation (CV%) was 12% (n = 27).

Paraoxonase 1 (PON1) activity

Paraoxonase activity of PON1 was assessed as the rate of formation of p-nitrophenol by hydrolysis of paraoxon. Activity was measured using 5 µl of freshly thawed serum from stored aliquots in 100 mM Tris- Cl buffer (pH 8) and CaCl2 containing 4 mM paraoxon. Nonenzymatic hydrolysis of paraoxon was subtracted from the rate of hydrolysis by serum. Activity was calculated using a molar extinction coefficient (E412) of 18,290 M − 1 cm − 1 and expressed in U/ml. One unit of the paraoxonase activity equals 1 nmol of p-nitrophenol produced/min/ml.

Phenylacetate was used as a substrate to study arylesterase activity of PON1. Rates of hydrolysis of phenylacetate was determined spectrophotometrically at 270 nm using diluted serum. Reaction buffer included 8 mM phenylacetate and 1 mM CaCl2 in 50 mM Tris HCl, pH 8.0. The amount of phenyl acetate hydrolyzed was calculated from the molar absorptivity, 1310 M-1cm-1. One unit of arylesterase activity is defined as 1 µmol of phenyl acetate hydrolyzed per minute per ml. For estimation of paraoxonase and arylesterase activity of PON1, all samples were run in triplicates.

Statistical analysis

Distribution pattern of the data for the variables was analyzed using Shapiro-Wilk test. Data with normal distribution were expressed as mean with standard deviation (SD) and nonparametric data were represented as median with interquartile values. For the categorical variables, results were presented in percentage. Student t-test and Mann-Whitney U test were performed to estimate the significance in the difference in HDL functional between two groups for parametric and non-parametric data respectively. Linear regression analysis was performed to test the association of cholesterol efflux capacity and antioxidative activity with apolipoprotein A-I. Correlation coefficient (r) and its corresponding p value were estimated using Pearson correlation coefficient separately and combined for cases (ACS) and controls.

Logistic regression was performed using control and ACS group as dependent variables and apolipoprotein A-I and HDL functions as independent variables. Odds ratio and their 95% confidence intervals (CI) were estimated per SD change in variable. Multivariate analysis was done where, in model 2, adjustment was done for cardiovascular risk factors like age, body mass index (BMI), gender, smoking, hypertension, diabetes and LDL. In model 3, additional adjustment for HDL -C level was done. Interaction between two continuous variables (i.e. HDL functions) was analyzed by running the logistic regression for interaction, margins and marginsplot commands were used for creating two-way contour to graph predictions from a model that includes an interaction between two continuous variables.

Two-sided test with p value < 0.05 was considered statistically significant. R Core Team (2018, R: A language and environment for statistical computing; R Foundation for Statistical Computing, Vienna, Austria; URL https://www.R-project.org/) software, Graph pad prism 6 (GraphPad software, California, USA) software, STATA 12.2/MP software and Adobe Illustrator (Adobe Systems, San Jose, CA, USA) software were used to perform the statistical analysis and creation of figures.

Results

Participants and biochemical parameters

The characteristics of the patients and healthy subjects and their biochemical parameters are summarized in the Tables 1 and 2 respectively. All ACS patients included in the study had ST segment elevation myocardial infarction (STEMI). There was a significant difference in the mean age of control group and ACS group ($p < 0.001$). There were more male subjects in ACS group than in control group. The ACS group had higher proportion of participants with diabetes and hypertension. They also had a higher percentage of smokers ($p < 0.0001$).

Table 1
Clinical characteristics of participants

Parameters	Control n = 110	ACS baseline n = 150	p value
Age (years)	43.6 ± 10.5	51.7 ± 11.4	< 0.001
BMI (kg/m^2)	25.1 ± 3.6	25.3 ± 3.5	ns
Gender Male/ Female n	65/45	134/18	
Smoking n (%)	10 (9)	83 (55)	< 0.001
Diabetics n (%)	13 (11.8)	42 (27.8)	< 0.001
Hypertensive n (%)	20 (18.1)	45 (29.8)	0.03

Data expressed as total (percentage). ACS, Acute coronary syndrome; BMI, Body Mass Index.

Table 2
Biochemical parameters of participants

Parameters	Control (n = 110)	ACS baseline (n = 150)	ACS follow-up (n = 100)
Apo A-I (mg/ml)	142.1 ± 43.3	112.5 ± 27.3***	127.2 ± 36.6###
Apo B (mg/ml)	72.3 ± 22.4	83.9 ± 28.9***	62.7 ± 20.5###
Total cholesterol (mg/dl)	161.2 ± 37.1	169.3 ± 42.0	137.0 ± 39.8###
HDL-C (mg/dl)	45.4 ± 9.6	40.2 ± 7.2***	38.3 ± 9.0
LDL-C (mg/dl)	104.0 ± 25.4	107.3 ± 34.9	80.8 ± 29.3
VLDL-C (mg/dl)	16.9 ± 7.2	20.6 ± 11.2*	15.8 ± 11.1###
TG (mg/dl)	117.3 ± 45.2	126.8 ± 67.8	134.6 ± 90.2
HDL function	**Control (n = 110)**	**ACS baseline (n = 150)**	**ACS follow-up (n = 100)**
Cholesterol efflux capacity (A.U.) Male Female	1.02 ± 0.16 1.04 ± 0.16 1.0 ± 0.14	0.88 ± 0.15*** 0.9 ± 0.14* 0.87 ± 0.15*	0.94 ± 0.17### 0.99 ± 0.19### 0.93 ± 0.19
Paraoxonase activity (U/ml) Male Female	105.1 ± 56.4 107.1 ± 56.2 101.9 ± 57.1	69.1 ± 38.8*** 69.5 ± 40.0* 65.5 ± 29.1*	81.1 ± 45.5### 82.4 ± 46.5### 72.1 ± 37.5
Arylesterase activity (U/ml) Male Female	112.0 ± 36.3 113.8 ± 38.8 109.4 ± 32.7	82.1 ± 27.9*** 81.0 ± 28.1* 90.2 ± 26.6*	101.8 ± 30.6### 100.4 ± 31.0### 111.8 ± 26.3###

Data are expressed as mean ± standard deviation. ACS, Acute coronary syndrome; Apo A-I, Apolipoprotein A-I; Apo B, Apolipoprotein B; HDL-C, HDL-cholesterol; LDL-C, LDL- cholesterol; VLDL-C, Very Low Density lipoprotein-cholesterol; TG, Triglycerides. *represents significance value for comparison between control and ACS patients. #represents significance value for comparison between ACS baseline and ACS follow-up patients. *p < 0.05; **p < 0.01; ***p < 0.001, #p < 0.05; ##p < 0.01; ###p < 0.001.

Patients presenting with ACS had significantly lower HDL-C (45.4 ± 9.6 vs 40.2 ± 9.6 mg/dl, p < 0.001) and apolipoprotein A-I (142.1 ± 43.3 vs 112.5 ± 27.3 mg/dl, p < 0.001) levels as compared to control subjects. ACS patients had significantly higher apo B levels as compared to controls. No significant difference was observed in total cholesterol (TC), LDL-C, very low density lipoprotein cholesterol (VLDL-C) and triglycerides (TG) between two groups overall.

HDL functions

We observed that ACS patients had significantly lower HDL cholesterol efflux capacity, 0.88 ± 0.15 Arbitrary Units (AU) in ACS and 1.02 ± 0.16 AU in control subjects ($p < 0.001$) (Fig. 1a). Arylesterase activity of PON1 was significantly lower in ACS patients (112.0 ± 36.3 vs 82.1 ± 27.9 U/ml, $p < 0.001$) (Fig. 1b). Paraoxonase activity of PON1 was significantly reduced in ACS patients (105.1 ± 56.4 U/ml in controls and 69.1 ± 38.8 U/ml in ACS subjects) (Fig. 1c).

Smokers with ACS had significantly lower arylesterase activity compared to non-smokers in ACS group (76.7 vs 88.7 U/ml, $p = 0.008$). HDL functions were also found to be significantly lower in male and female ACS subjects compared to male and female controls respectively (Table 3).

HDL function	Control (n =110)	ACS baseline (n = 150)	ACS follow-up (n = 100)
Cholesterol efflux capacity (A.U.)	1.02 ± 0.16	0.88 ± 0.15***	0.94 ± 0.17###
Male	1.04 ± 0.16	0.9 ± 0.14*	0.99 ± 0.19###
Female	1.0 ± 0.14	0.87 ± 0.15*	0.93 ± 0.19
Paraoxonase activity (U/ml)	105.1 ± 56.4	69.1 ± 38.8***	81.1 ± 45.5###
Male	107.1 ± 56.2	69.5 ± 40.0*	82.4 ± 46.5###
Female	101.9 ± 57.1	65.5 ± 29.1*	72.1 ± 37.5
Arylesterase activity (U/ml)	112.0 ± 36.3	82.1 ± 27.9***	101.8 ± 30.6###
Male	113.8 ± 38.8	81.0 ± 28.1*	100.4 ± 31.0###
Female	109.4 ± 32.7	90.2 ± 26.6*	111.8 ± 26.3###

Data are expressed as mean ± standard deviation. ACS, Acute coronary syndrome; *represents significance value for comparison between control and ACS subjects. #represents significance value for comparison between ACS baseline and ACS follow-up subjects. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, #$p < 0.05$; ##$p < 0.01$; ###$p < 0.001$.

Table 3

Comparison of HDL functions

HDL functions in ACS patients at follow-up

The follow-up samples from 100 patients were available. All ACS patients enrolled in the study were statin naïve and were on high dose statin after they were diagnosed with ACS. 45 were lost to follow-up and 5 had a second event of ACS or had died. A significant decrease in body mass index was observed after six months follow up (25.69 ± 3.9 vs 24.7 ± 4.0 kg/m^2, $p < 0.001$). In the study, out of 100 ACS patients who were followed up, 62 were smokers at presentation and out of these 62, 37 quit smoking by the end of follow up.

Change in lipid profile after therapy is summarized in Table 2. A significant reduction was observed in total cholesterol, VLDL cholesterol and LDL-C levels after therapy. HDL-C also decreased after treatment, but the decrease was not significant; however, there was a significant increase in apolipoprotein A-I levels. Triglyceride levels also decreased after therapy, but the decrease was not significant. Apolipoprotein B levels were reduced after six months. The patients at follow-up had significantly lower total cholesterol, apo B and LDL-C levels compared to control subjects. They also had significantly lower apo A-I levels and HDL-C levels.

We observed a significant increase in cholesterol efflux capacity at follow up after six months in ACS patients (0.89 ± 0.14 vs 0.98 ± 0.17 AU; $p < 0.001$). The efflux capacity of ACS follow-up and control subjects were found to be similar (Fig. 1a). A significant improvement in PON1 arylesterase (Baseline: 85.4 ± 30.2; Follow up: 101.8 ± 30.6 U/ml; $p < 0.001$) (Fig. 1b) and paraoxonase activity (Baseline: 69.1 ± 42.; Follow-up 81.1 ± 45.5 U/ml; $p < 0.001$) (Fig. 1c) was observed after six months of therapy. Although there was marked increase in paraoxonase and arylesterase activity of ACS patients after statin therapy, it was still significantly lower compared to control subjects.

Correlation between HDL function and apolipoprotein A-I

Linear regression analyses showed a significant positive correlation between cholesterol efflux capacity and apolipoprotein A-I ($r = 0.39$. $p = 0.001$), and a similar correlation was observed in control ($r = 0.29$, $p = 0.0079$), ACS baseline ($r = 0.3$, $p = 0.0013$) (Fig. 2a) and ACS follow-up ($r = 0.26$, $p = 0.01$) groups separately (Fig. 2d). However, no correlation was observed between CEC and HDL-C levels in control or ACS group.

A strong positive correlation was observed between arylesterase activity and the levels of apolipoprotein A-I in controls ($r = 0.3$, $p = 0.0012$), ACS baseline ($r = 0.19$, $p = 0.019$) (Fig. 2b) and ACS follow-up groups ($r = 0.26$, $p = 0.007$). Figure 2e). We did not observe any correlation between paraoxonase activity and apolipoprotein A-I levels and HDL- C levels (Fig. 2c)

hs-CRP levels were measured as an index of inflammation in acute coronary syndrome subjects. Cholesterol efflux capacity showed an inverse correlation with hs-CRP levels at follow-up ($r = -0.2$, $p = 0.03$) and the same trend was also observed in the baseline ACS data ($r = -0.2$, $p = 0.03$) (Fig. 2f).

Association of HDL functions with acute coronary syndrome

Logistic regression was performed to analyze the association of apolipoprotein A-I, cholesterol efflux capacity and PON1 activity of HDL with the risk of ACS (Fig. 3). Apolipoprotein A-I, Cholesterol efflux capacity, PON1 paraoxonase and arylesterase activities were seen to have a protective effect (Model 1). The effect of apolipoprotein A-I, efflux capacity and antioxidative activity remained significant even after adjustment for age, gender, BMI and other cardiovascular risk factors like smoking, diabetes, hypertension and LDL-C (Model 2) (Fig. 3). Higher cholesterol efflux capacity (odds ratio per 1-SD increase: 0.49; 95% confidence interval: 0.29–0.8; $p = 0.006$), PON1 paraoxonase activity (odds ratio per 1-SD increase: 0.44; 95% confidence interval: 0.28–0.66; $p = 0.0002$) and arylesterase activity (odds ratio per 1-SD: 0.50; 95% confidence interval: 0.34–0.72; $p = 0.003$) were associated with lower odds of development of ACS even after additional adjustment for HDL-C levels (Model 3).

We then assessed interactions between HDL functions (cholesterol efflux capacity and arylesterase activity, cholesterol efflux capacity and paraoxonase activity) to predict the probability of ACS. This interaction is meant to represent how the effect of CEC on the predicted probability of ACS differs across levels of ARE and vice versa (Fig. 4a). Similarly, the effect of CEC on the predicted probability of ACS at different levels of PON was also analyzed as in Fig. 4b. We calculated the predicted probability of acute coronary syndrome for all combinations of cholesterol efflux capacity ranging from 0.5 to 1.5 with an increment of 0.5, and arylesterase activity, ranging from 20 to 210 U/ml (increment 50). Similarly, we also analyzed the interaction of CEC with paraoxonase activity (ranging from 40 to 240 U/ml with an increment of 10) We observed that an individual with cholesterol efflux capacity (CEC) 1 A.U. and arylesterase activity (ARE) 100 U/ml has a 50% chance of having acute coronary syndrome (ACS) ($p < 0.001$), while an individual with CEC 1 A.U. and ARE 190 U/ml has a 10% chance of having ACS ($p = 0.06$). We found that the interaction between two HDL functions was significant for lower values of cholesterol efflux capacity and PON1 activity (arylesterase activity and paraoxonase activity) but was not significant for low cholesterol efflux values and high arylesterase or paraoxonase values.

Discussion

In patients presenting with ACS, HDL functions (cholesterol efflux capacity and antioxidative activity) were significantly impaired. Impaired HDL function was found to be associated with risk of having ACS even after adjusting for known risk factors including HDL-C levels. At follow-up, there was a significant improvement in the HDL function in these patients despite a marginal decrease in HDL-C levels, though PON1 paraoxonase and arylesterase activity remained lower than in controls. Cholesterol efflux capacity and arylesterase activity were significantly correlated with apolipoprotein A-I levels but not with HDL-C levels. We also found that combination of cholesterol efflux capacity and antioxidative activity strengthened the probability of ACS prediction.

CEC, which represents HDL's ability to prevent the formation of macrophage foam cells by removing excess cholesterol from macrophages, rather than HDL cholesterol levels, has been shown to have significant negative correlation with atherosclerotic burden [8, 13]. Our results demonstrate that HDL from ACS patients had lower ability to mediate cholesterol efflux from lipid laden macrophages compared to HDL from healthy subjects. These results are consistent with the results obtained in other studies that compared the cholesterol efflux capacity in ACS immediately after the onset of symptoms [3, 10].

PON1 is an HDL associated anti-atherosclerotic enzyme that prevents the accumulation of lipoperoxides and inhibits the lipid oxidation in low-density lipoproteins (LDL), an observation supported by earlier studies [11, 14, 25]. The binding of PON1 to apolipoprotein A-I in HDL modulates its activity [18]. In the present study, we observed lower PON1 arylesterase and paraoxonase activities in ACS group which improved at follow-up but was lower than controls suggesting decreased activity despite optimal therapy.

Our group of acute coronary syndrome patients had a high percentage of smokers and since smoking is one of the risk factors for ACS, we compared the functionality of HDL between smokers and nonsmokers presenting with ACS. We observed a lower HDL efflux capacity and arylesterase activity in smokers presenting with ACS than non-smokers who had ACS. This suggests that oxidative stress induced by smoking to be a major factor for impaired HDL function.

Evidence from scientific literature indicates that long term statin therapy decreases the progression of atherosclerotic plaque and risk of major cardiovascular events in ACS [19]. We found a significant reduction in LDL cholesterol, total cholesterol, VLDL cholesterol and a statistically insignificant decrease in HDL cholesterol.

We observed that the inflammatory status improved after therapy as indicated by decrease in hs-CRP by 62%, an effect that could partially be contributed by the statin component of the therapy. In the present study, while a significant improvement and increase was observed in cholesterol efflux capacity and PON1 activity after six months of treatment, the latter continued to be significantly lower than in normal participants even after six months.

A study on association of HDL cholesterol efflux capacity with incidence of coronary artery disease using radiolabeled cholesterol reported that the cholesterol efflux capacity was positively associated with apolipoprotein A-I levels and inversely associated with the incidence of coronary disease [21]. Data from our efflux assay performed using BODIPY- cholesterol loaded THP-1 cells, found a similar association between cholesterol efflux capacity and risk of ACS. Other functional parameters of HDL like ARE and PON1 activity were also shown to be protective against development of ACS. CEC of apo B depleted serum and apolipoprotein A-I levels in both control and ACS patients (baseline and follow-up) showed significant correlation and 15% of the variability in cholesterol efflux capacity of HDL could be explained by apolipoprotein A-I levels.

However, we found no correlation between circulating HDL cholesterol levels and cholesterol efflux capacity in control subjects and ACS patients. Furuyama F et al. in their study on effect of cardiac rehabilitation on HDL function have also shown significant correlation of CEC and ARE with apoA-I levels rather than HDL-cholesterol levels [7]. This may be because cholesterol content makes up only about 20%

of the HDL particle and even that proportion differs between different HDL particles. This supports the concept that HDL cholesterol level is not an accurate measure of HDL function and marker of CVD risk as seen in recent studies [26]. Therefore, establishing a standardized cell free system for measuring CEC will help clinicians to not only understand the association of cholesterol efflux capacity with cardiovascular disease but also help with identification of high-risk individuals for intervention.

Currently cardiovascular risk stratification is done using parameters like HDL-C and apoA1 levels. The interaction observed between HDL functions suggests that impairment of both the HDL functions increases the chances of having ACS, while high CEC, ARE and PON values confer protection against ACS. Also, our results indicate that the risk prediction for ACS can be improved by measuring the HDL functional parameters together.

Indians have a high prevalence of low HDL-C levels and cardiovascular disease. However, HDL function has not been studied in them and this is the first study to do so. Whether low HDL function confer additional risk of CVD cannot conjecture since comparative efflux capacity to that of the Caucasian population is not available and no standardized reference values are available for the same. However, future studies will be useful to see this aspect of CVD risk.

Our study has a few limitations. Two groups were not age and gender matched but we have adjusted for these two factors in our analysis. We evaluated the levels of only one inflammatory marker although other inflammatory and oxidative stress markers are also seen elevated in case of acute coronary syndrome [2] and are responsible for the generating dysfunctional HDL [24]. Finally, while the results prove that HDL function is a good predictor for risk of ACS, significant improvement is required in developing a composite measure of HDL function that is adaptable for clinical set up.

The correlation of HDL functions with apolipoprotein A-I along with recent evidence supports the rationale of HDL functions being considered as the predominant therapeutic target for lipid lowering therapy rather than simply its cholesterol mass [6]. Current studies are focused on improving the functions of HDL particles, with one of them, CSL-112, a recombinant apolipoprotein A-I undergoing Phase 3 clinical trials in patients with myocardial infarction to see the effect on major adverse cardiac events (MACE), after Phase 2b trials having shown large increases in efflux capacity with this drug [5, 15]. Our data support the rationale of therapies to improve HDL function to manage the residual risk observed even after optimal decreases in LDL levels in patients with ACS.

Conclusion

In conclusion, our study supports the concept of HDL function as a superior measure of cardiovascular risk than measuring HDL-cholesterol levels. Standard therapy after ACS improved HDL function partially suggesting remaining residual risk in these patients which maybe future targets of therapy. Our findings also indicate that using a combination of HDL functions (cholesterol efflux capacity and antioxidative activity) could improve the predictive accuracy for ACS.

Abbreviations

ACS: Acute coronary syndrome

Apo A-I: apolipoprotein A-I

Apo B: Apolipoprotein B

ARE: Arylesterase

CEC: cholesterol efflux capacity

CVD: Cardiovascular disease

HDL-C: high density lipoprotein- cholesterol

hsCRP: high sensitive C reactive protein

LDL-C: Low density lipoprotein – Cholesterol

PON1: Paraoxonase1

TC: Total Cholesterol

TG: Triglyceride

VLDL: Very low density lipoprotein – Cholesterol

Declarations

Ethics approval and consent to participate

The study protocol was approved by Institute Ethics Committee of All India Institute of Medical Sciences, New Delhi (Reference Number: IESC/T-380/17.10.2014). Signed informed consent was obtained prior to enrolling in the study. The participants did not receive any stipend for taking part in the study.

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

None of the authors have any conflict of interest to declare.

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Authors contribution

AS contributed towards the design and performance of the study, analysis of data, and preparation of the manuscript. HT contributed towards the design and performance of the experimental procedures, analysis of data, and preparation of the manuscript. VV contributed towards the design and performance of the study, analysis of data, and preparation of the manuscript. AR and SS contributed towards the design of the study, sample collection, analysis of data, and preparation of the manuscript. LR contributed towards the design of the study, assessment of lipid profile, analysis of data, preparation of the manuscript. MK contributed towards the design and performance of the study, analysis of data, and preparation of the manuscript. All authors were involved in interpretation of data and the final approval of the manuscript.

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Figures

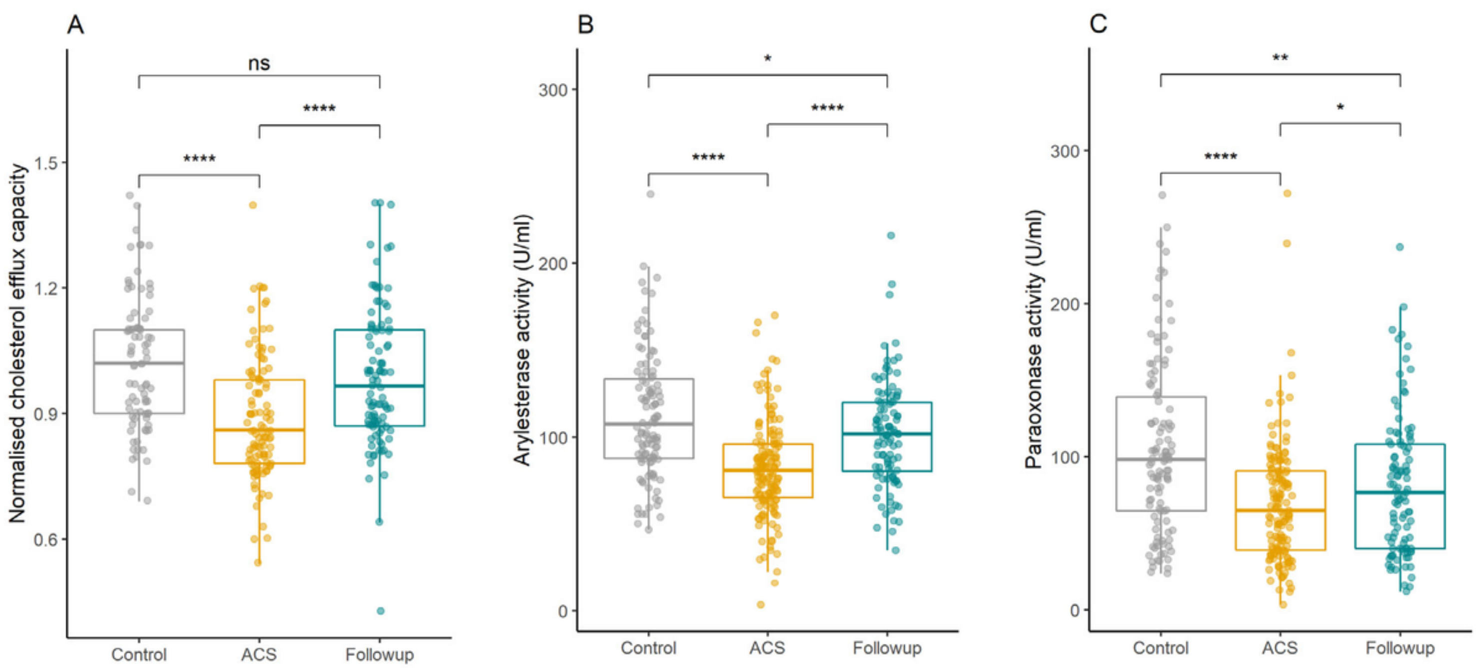


Figure 1

HDL functions in controls, ACS baseline and ACS follow-up. HDL mediated cholesterol efflux capacity measured using BODIPY-cholesterol loaded THP-1 macrophages (a), Arylesterase activity (b), and Paraoxonase activity (c) of PON1 in control and ACS patients at baseline and follow-up. Statistical analysis to determine the difference between two groups was performed using student t test. Data are shown as mean ± SD. Stars represents significance. *p<0.05, **p<0.01, ****p<0.0001. ns represents non-significant

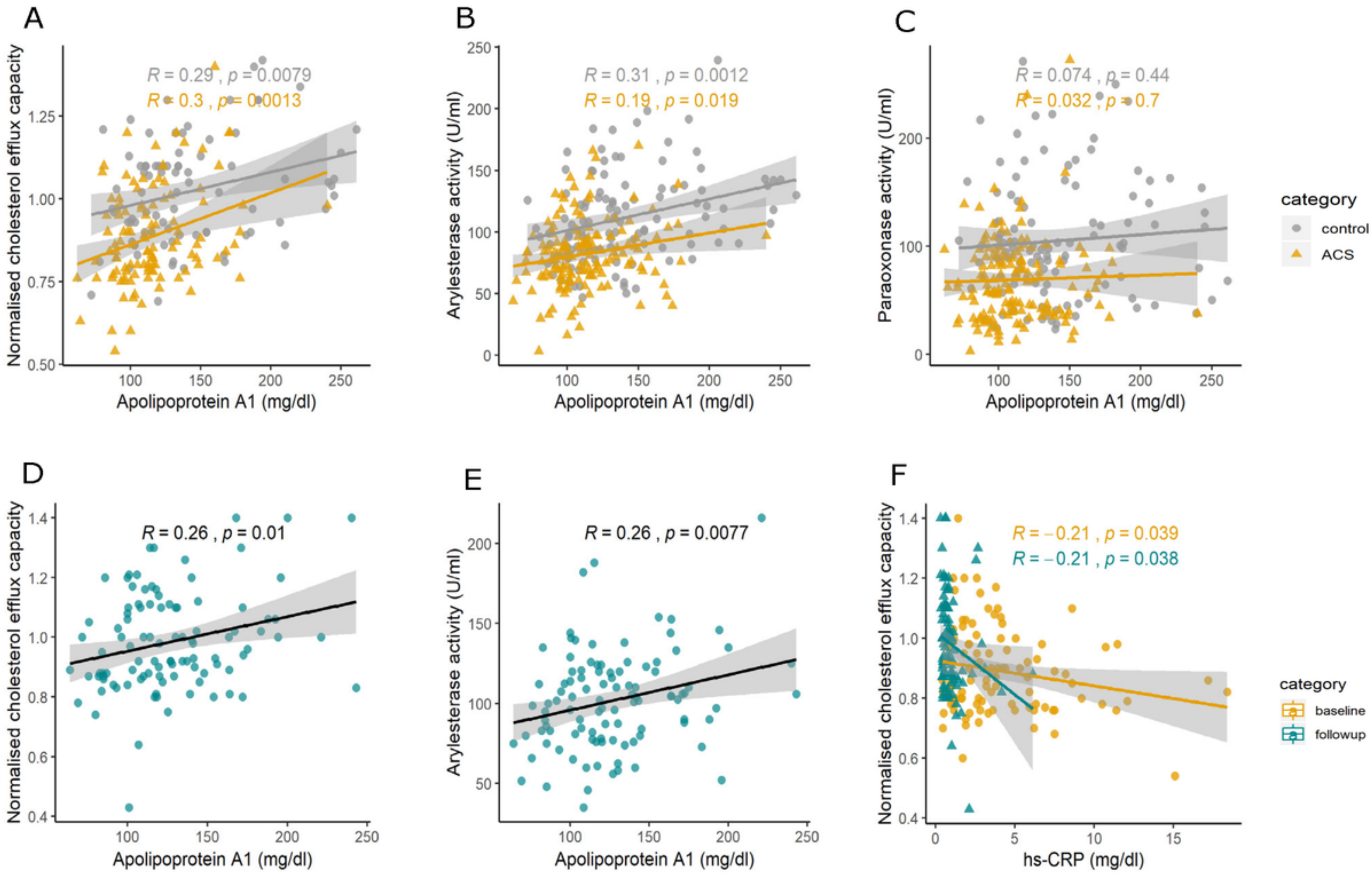
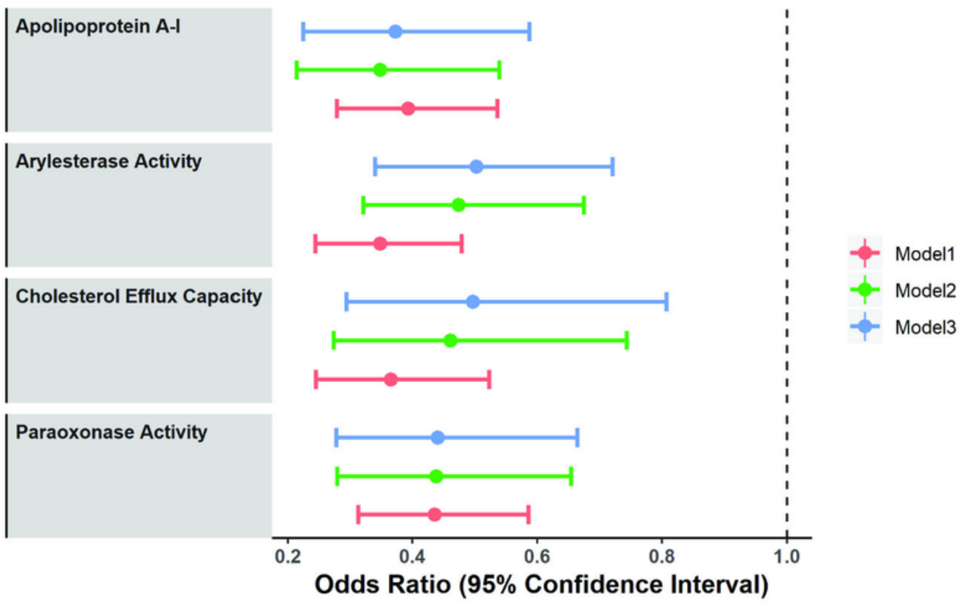


Figure 2

Correlation of HDL functions with apolipoprotein A-I. Correlation of (a) cholesterol efflux capacity and (b) Arylesterase activity, (c) paraoxonase activity of PON1 with apolipoprotein A-I in control, ACS subjects at baseline and follow-up (d, e). Correlation of (f) cholesterol efflux capacity with hs-CRP levels in ACS patients at baseline and follow-up. Pearson correlation coefficient (R) and p value are shown. Line represents the regression line



Odds Ratio/SD increment (95% CI)	p-value
0.37 (0.22-0.59)	<0.0001
0.34 (0.21-0.54)	<0.0001
0.39 (0.28-0.53)	<0.0001
0.50 (0.34-0.72)	0.0003
0.47 (0.32-0.68)	<0.0001
0.35 (0.25-0.48)	<0.0001
0.49 (0.29-0.8)	0.006
0.46 (0.27-0.74)	0.002
0.37 (0.25-0.52)	<0.0001
0.44 (0.28-0.66)	0.0002
0.44 (0.28-0.65)	0.0001
0.43 (0.32-0.59)	<0.0001

Figure 3

Association of HDL functions with acute coronary syndrome. Odds ratios per 1-SD increase their respective 95% confidence intervals (CIs) and p values are shown for acute coronary syndrome. Dots represent odds ratios, and error bars indicate 95% confidence intervals. Logistic regression model 1 indicates unadjusted odds ratio, model 2 adjusted for age, gender, BMI, smoking status, diabetes, hypertension and LDL. In model 3, additional adjustment for HDL was done

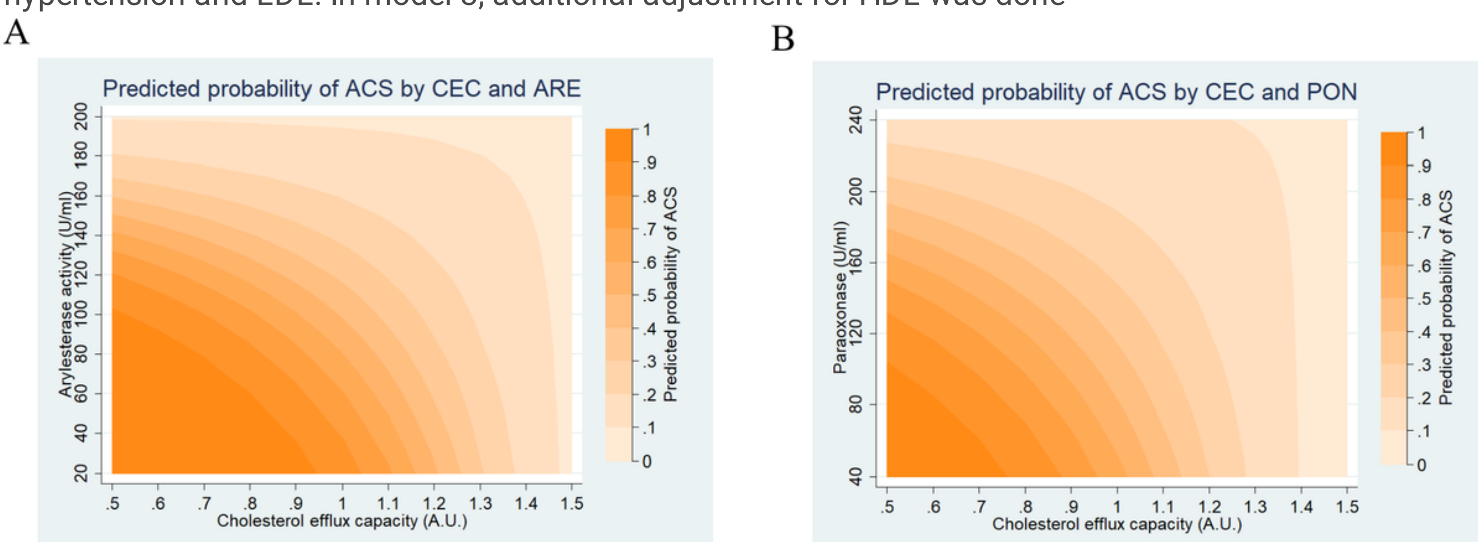


Figure 4

Predicted probability of Acute coronary syndrome. Predicted probability based on interaction between cholesterol efflux capacity (CEC) and (a) Arylesterase activity (ARE) and (b) Paraoxonase activity (PON)