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Carotid intima media thickness and low high-density lipoprotein (HDL) in South Asian immigrants: could dysfunctional HDL be the missing link?

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Abstract

Introduction: South Asian immigrants (SAIs) in the US exhibit higher prevalence of coronary artery disease (CAD) and its risk factors compared with other ethnic populations. Conventional CAD risk factors do not explain the excess CAD risk; therefore there is a need to identify other markers that can predict future risk of CAD in high-risk SAIs. The objective of the current study is to assess the presence of sub-clinical CAD using common carotid artery intima-media thickness (CCA-IMT), and its association with metabolic syndrome (MS) and pro-inflammatory/dysfunctional HDL (Dys-HDL).

Material and methods: A community-based study was conducted on 130 first generation SAIs aged 35–65 years. Dys-HDL was determined using the HDL inflammatory index. Analysis was completed using logistic regression and Fisher's exact test.

Results: Sub-clinical CAD using CCA-IMT ≥ 0.8 mm (as a surrogate marker) was seen in 31.46%. Age and gender adjusted CCA-IMT was significantly associated with type 2 diabetes (p = 0.008), hypertension (p = 0.012), high-sensitivity C-reactive protein (p < 0.001) and homocysteine (p = 0.051). Both the presence of MS and Dys-HDL was significantly correlated with CCA-IMT, even after age and gender adjustment. The odds of having Dys-HDL with CCA-IMT were 5 times (95% CI: 1.68, 10.78).

Conclusions: There is a need to explore and understand non-traditional CAD risk factors with a special focus on Dys-HDL, knowing that SAIs have low HDL levels. This information will not only help to stratify high-risk asymptomatic SAI groups, but will also be useful from a disease management point of view.

Key words: coronary artery disease, risk factors, common carotid artery intima media thickness, South Asians, dysfunctional high-density lipoprotein.

Introduction

Assessment of coronary artery disease (CAD) risk relates to the availability of effective treatments that inhibit the development and progression of atherosclerosis. Effective therapeutic intervention has advanced the concept of primary prevention of CAD. Primary prevention focuses on identifying asymptomatic individuals without a prior history of CAD, who are at sufficiently high risk for a future CAD event to justify gradually more intensive risk reduction efforts [1]. The concept of risk assessment

was first introduced by the Framingham Heart Study (FHS), and has been expanded upon in the decades since [2]. Risk factors routinely used in risk profiles or algorithms include age, blood pressure, serum cholesterol (high-density lipoprotein (HDL) and low-density lipoprotein (LDL)), type 2 diabetes (T2D), and cigarette smoking. Others are being added or considered frequently.

Assessment of common carotid artery intima-media thickness (CCA-IMT) is well recognized in diagnosing atherosclerosis [3]. With the use of high-resolution B-mode ultrasonography, one can evaluate early carotid atherosclerosis precisely and noninvasively. Of the various noninvasive imaging methods available, CCA-IMT measurement obtained with B-mode ultrasound is currently the only method recommended by the American Heart Association (AHA) for inclusion in the evaluation of risk [2]. Moreover, CCA-IMT is also used as a noninvasive end point in epidemiological studies and clinical trials to gauge progression and regression of atherosclerosis [4, 5]. Furthermore, CCA-IMT has been used recently not only as a surrogate end point for atherosclerosis of CAD but also as a good indicator of the presence and extent of CAD [6].

Even though CAD event rates have decreased by 50% in the US and other developed countries, event rates in South Asians (people with ancestors from the Indian subcontinent, i.e. India, Pakistan, Bangladesh, Nepal, Bhutan, and Sri Lanka) have doubled in the past two decades [7]. South Asian immigrants (SAIs) exhibit higher prevalence of CAD and its risk factors as compared with Caucasians (10% vs. 2.5%) [8, 9], and these findings are not limited to the US but appear to be a global phenomenon [10]. Although South Asians represent the second fastest growing Asian immigrant population in the US, little is known regarding their increased risk for CAD [7]. In general, the notion that the immigrant population carries higher risk for CAD also holds true for SAIs. As a whole group, SAIs, compared to other populations, have much higher prevalence of T2D, metabolic syndrome (MS), insulin resistance, central obesity, dyslipidemias [lower HDL, increased lipoprotein a (Lp[a]), higher triglycerides (TGs)], increased thrombotic tendency, and low levels of physical activity [11–13]. Furthermore, CAD risk factors are present at a younger age in South Asians compared to other populations, resulting in CAD at a younger age than in other populations [13]. However, even taking these differences into account, conventional risk factors, insulin resistance parameters, or MS, although important in predicting CAD risk, may not fully account for the increased risk in SAIs [12]; thus, a search for additional markers is warranted, to promote early detection and prevention of CAD in this high-risk group.

Among numerous genetic and lifestyle parameters, dyslipidemias are among the most prominent risk factors for CAD. The HDL plays a protective role in preventing CAD, and low HDL is an independent risk factor for CAD [14]. This protective effect of HDL is related to its role as an antiatherogenic agent that prevents LDL oxidation. According to several recent Caucasian studies, in patients with CAD, HDL was found not only ineffective as an antioxidant but, paradoxically, appears to be a pro-oxidant, as assessed by its lipid peroxide content [15–17]. This pro-inflammatory HDL, named as dysfunctional HDL (Dys-HDL), accumulates oxidants that inhibit HDL-associated antioxidant enzymes, render apolipoprotein A-I (ApoA-I), the major protein of HDL, unable to promote ABCA1 mediated cholesterol efflux, and promotes the formation of LDL-derived oxidized lipids. According to National Cholesterol Education Program (NCEP) ATP III guidelines, an HDL level < 40 mg/dl is defined as an independent risk factor for CAD and low HDL is often present in high-risk patients with CAD [18]. Current data indicate that a 1% increase in HDL serum concentration can reduce CV risk by 2–3%, independent of LDL levels [19]. However, recent studies in animals and humans have demonstrated that the protective effects of HDL are better correlated with HDL function than HDL cholesterol levels (ref). We recently found that 50% of participants carrying dysfunctional HDL (Dys-HDL) without CAD showed a significant association with lipoprotein [a], low HDL and CCA-IMT (age-adjusted) [19]. Increased CCA-IMT measurements have been strongly associated with an increased risk of cardiovascular morbidity and mortality, as well as a marker of atherosclerosis regression and dyslipidemia improvement in patients on lipid-lowering therapy [6]. However, whether CCA-IMT relates to Dys-HDL, MS, and other CAD risk factors in SAIs is not known.

The objective of the current study was to determine the association of sub-clinical CAD using CCA-IMT as a surrogate marker of atherosclerosis in SAIs with Dys-HDL and other CAD risk factors.

Material and methods

In this cross-sectional pilot study, SAIs (South Asian Indians) between the ages of 35 and 65 years were recruited from the main Hindu temples in the states of Georgia and Kansas. We chose this age because CAD and its risk factors occur at younger ages in SAIs as compared to other populations [8]. The SAI population in the US is most readily accessed through their temples of worship. Therefore, although study information was made available and distributed using different methods, the majority of the study subject recruitment was done through Hindu temples. This approach

was used because there is no national level census or data available on South Asians providing a correct estimate of the total population within the US. Therefore, we understand that the results of this study may not be generalizable, although most SAIs visit temples on weekends, representing several ethnic groups. Study information was made available by distributing flyers in the temples and announcements through local newspapers outlining the purpose, rationale, and design of the study. Written informed consent and ethics approval was obtained from study subjects and the university institutional review board respectively. Information on socio-demographic status, ethnicity (based on spoken language), personal lifestyle characteristics, and both traditional and non-traditional risk factors for CAD was obtained. Twelve-hour fasting blood samples were collected for measurements of high-sensitivity C-reactive protein (hsCRP), total lipid testing including total cholesterol, triglycerides (TGs), HDL, low-density lipoprotein (LDL), and lipoprotein a (Lp[a]). Insulin, fibrinogen, homocysteine and ApoA-I serum levels were also measured.

Carotid ultrasound Doppler for common carotid intima-media thickness (CCA-IMT)

The CCA-IMT is defined by Pingoli *et al.* as the distance from the leading edge of the lumen-intima interface of the far wall to the leading edge of the media-adventitia interface of the far wall [20]. B-mode ultrasound scanning of bilateral CCAs was performed by a trained non-invasive vascular ultrasound technician at the University of Kansas Medical center study clinics, using a Sonosite MicroMaxx ultrasound machine (Sonosite, Inc Bothell, WA) with a 10.0 MHz linear array transducer. Both CCAs were scanned in supine position. A total of eight images were obtained (four on each side), 1 cm proximal to the carotid bulb, using a posterior wall (far wall) approach. ECG leads were placed to obtain end-diastolic measurements. Images were recorded and stored on a disk. The CCA-IMT approach for IMT measurements was preferred because the CCA-IMT is reproducible and predictive of future cardiovascular events, and the data collection is more complete than other non-invasive markers [21]. Measurements of the internal carotid and bifurcation segments tend to have many more missing values. The Mannheim Intima-Media Thickness Consensus suggested that measurement of the CCA is ideal [22].

Any focal thickening of the intima-media complex or carotid plaque was not included in the analysis. Two cardiologists, who were blinded to participants' identities and clinical information, analyzed stored images by using the SonoCalc IMT software. Measurement of the far wall of the carotid artery was preferred, since studies comparing ultrasound measurements with histology suggest that far-wall CCA-IMT measurements are more indicative of the true thickness of the arterial wall [21, 22]. Near-wall CCA measurements, in comparison, are limited by their dependence on the axial resolution and gain settings of the equipment used and show greater variation between repeated measurements [23]. Participants with values greater than 0.8 mm were considered to be IMT positive. Previous epidemiological studies suggest that a value of IMT at or above 0.8 mm is associated with a significantly increased absolute risk of CAD [23]. In this study a CCA-IMT value of 0.8 mm or more was considered abnormal. CCA-IMT values were adjusted for age as age can influence IMT [23]. We did not include carotid plaque in this study.

Assessment of dysfunctional HDL (Dys-HDL)

The diagnosis of Dys-HDL has historically been made with a cell-based assay that requires endothelial cells, smooth muscle cells, and monocytes. However, the use of a cell-based assay is not practical for large-scale studies. A cell-free assay has been developed to detect HDL that is dysfunctional [24, 25]. The details on Dys-HDL assessment using a cell-free assay have been published previously [19]. Briefly, this is a rapid test for HDL function that does not require cells and gives results highly comparable to those of the previously described cell-based assay. The HDL was isolated from blood samples using dextran sulfate precipitation. The LDL, necessary in the cell-free assay for testing the ability of HDL to protect against LDL oxidation, was prepared from a normal donor and was aliquoted and cryo-preserved in sucrose. Dichlorofluorescein-diacetate (DCFH-DA) was dissolved in fresh methanol at 2.0 mg/ml, incubated at room temperature, and protected from light for 30 min, which resulted in the release of dichlorofluorescein (DCFH) that produced an intense fluorescence upon interaction with oxidized lipid. Fluorescence was determined using a plate reader (Spectra Max, Gemini XS; Molecular Devices) at an excitation wavelength of 485 nm, an emission wavelength of 530 nm, and a cutoff of 515 nm with the photomultiplier sensitivity set at medium. For this study, the coefficient of variation for this assay was 9.6% [26]. Similarly, the HDL-inflammatory index (HII) was calculated by normalizing the cell-free assay values obtained for LDL alone as 1.0 [26]. If the addition of a test HDL resulted in a value of 1.0 or greater, the test HDL was classified as pro-inflammatory (dysfunctional). Conversely, if the addition of the standard normal LDL together with a test HDL resulted in a value less than 1.0, the test HDL was classified

as anti-inflammatory. To support the results of the cell-free assay, we also measured serum hsCRP.

Data analysis and power calculation

We enrolled 130 first generation SAIs, with different ethnic backgrounds. This is a pilot study survey with a fixed sample of 130 participants to assess the Dys-HDL and sub-clinical CAD using CCA-IMT as a surrogate marker for atherosclerosis. The power calculation for assessing Dys-HDL was based on a χ^2 contingency table analysis [dysfunctional HDL (Yes/No) vs. CCA-IMT (Yes/No)], based on the available data on CCA-IMT in South Asians (SAs) [19, 27]. Assuming that 20% of 130 have CCA-IMT, we have 91% power at the 5% α level and 85% power at the 1% α level to detect the Dys-HDL difference between two groups.

Statistical analysis

Baseline socio-demographic characteristics and CAD risk factors were summarized by frequency distributions and percentages for qualitative measures and means and standard deviations for quantitative measures. Maximum likelihood estimates and asymptotic 95% confidence intervals were calculated for the prevalence of disease/diagnosis outcome measures. Bivariate tests of association and odds ratios were performed by simple logistic regression. Multiple logistic regression models were used to assess the relative importance of variables found to be significantly associated with the outcome from the bivariate assessments. All statistical tests were two-sided and performed at the 0.05 level of significance.

Results

Out of 130 subjects, complete information was obtained on 129, who thus constituted our study sample. The study subjects' characteristics are shown in Table I. The mean age of subjects was 51 ±9.23 years with almost equal numbers of males and females. The study group presented a homogeneous mixture of various ethnicities (based on spoken language and ethnic background) including Hindi speaking (18%), Gujaratis (18%), and South Indians (26%). More than 50% received up to postgraduate level education. Significant prevalence of CAD risk factors (Table II) was observed: (a) hypertension – 45%, (b) high cholesterol ≥ 200 mg/dl – 41.6%, (c) T2D – 34.47%, (d) HDL < 40 mg/dl – 26.4%, (e) LDL ≥ 150 mg/dl – 16.9%, (f) Lp[a] – 35.7%, (g) hsCRP (≥ 5) – 48.74%, (h) BMI ≥ 23 – 78.4%, (i) obesity (BMI ≥ 30) –18.2%; (f) family history of CAD and T2D was 34.4% and 48.4% respectively. 82.8% were physically active. Sub-clinical CAD using CCA-IMT ≥ 0.8 mm (as a surrogate marker) was seen in 31.46%. Increased obesity is also reflected in increased waist circumferences in both genders in this study (Table II). Based on the Internal Diabetes Federation (IDF) definition, MS was seen in 29.7% of SAIs without CAD (Table II).

CCA-IMT association with CAD risk factors

The association of CCA-IMT with CAD risk factors was assessed using Fisher's exact test. Age and gender adjusted CCA-IMT was significantly associated with T2D (p = 0.008), hypertension (p = 0.012), hsCRP (p < 0.001) and homocysteine (p = 0.051).

Dys-HDL, MS and CCA-IMT

Dys-HDL was measured by the HDL inflammatory index using the cell-free assay [25]. Twenty-six percent (26.05%) had HDL inflammatory index ≥ 1, suggesting pro-inflammatory HDL or Dys-HDL.

Table I. Socio-demographic characteristics of study sample (n = 129)

Variable	Result
Age, means ± SD [years]:	51.30 ±9.23
Male	51.04 ±9.64
Female	51.68 ±8.70
Gender, *n* (%):	
Male	76 (58.6)
Female	53 (41.4)
Ethnicity, *n* (%):	
South Indian	33 (25.6)
Gujarati	23 (17.8)
Hindi	23 (17.8)
Bengali	10 (7.8)
Punjabi	8 (6.2)
Other	32 (26.2)
Work type, *n* (%):	
Employee full time	91 (70.5)
Homework	14 (10.9)
Employee part time	9 (7.0)
Unemployed	6 (4.7)
Other	8 (6.2)
Education, *n* (%):	
Postgraduate	67 (51.9)
Graduate	30 (23.3)
Undergraduate	24 (18.6)
Other	1 (0.8)

Table II. Coronary artery disease (CAD) risk factors and markers (n = 129)

Variable	*N* (%)	Mean ± standard deviation
BMI [kg/m^2]:		26.37 ±5.08
Normal (< 23)	27 (21.62)	21.84 ±1.68
Overweight (23–30)	76 (60.14)	25.82 ±2.05
Obese (≥ 30)	22 (18.24)	34.07 ±6.45
Total LDL [mg/dl]:		117.63 ±35.61
Normal (< 150)	103 (83.06)	106.08 ±24.73
Abnormal (≥ 150)	21 (16.94)	174.95 ±24.44
Total HDL [mg/dl]:		48.38 ±10.99
Normal (> 40)	92 (73.6)	52.95 ±8.98
Abnormal (≤ 40)	33 (26.4)	35.79 ±4.31
Dys-HDL [mg/dl]:		0.83 ±0.74
Normal (< 1.0)	88 (73.95)	0.53 ±0.17
Dysfunctional (≥ 1.0)	31 (26.05)	1.71 ±1.02
Total cholesterol [mg/dl]:		193.17 ±38.97
Normal (< 200)	73 (58.4)	167.74 ±22.34
Abnormal (≥ 200)	52 (41.6)	229.31 ±27.37
Triglycerides [mg/dl]:		160.44 ±114.56
Normal (< 150)	73 (58.4)	99.23 ±26.88
Abnormal (≥ 150)	52 (41.6)	246.90 ±134.70
Lipoprotein [a] [mg/dl]:		13.61 ±18.99
Normal (< 10)	79 (64.23)	4.59 ±1.79
Abnormal (≥ 10)	44 (35.77)	30.02 ±24.46
Apo lipoprotein A-1:		150.36 ±31.94
Normal (94–176 mg/dl)	95 (76.0)	142.06 ±22.19
Abnormal (other values)	30 (24.0)	178.13 ±41.30
hsCRP [mg/l]:		3.32 ±2.56
Normal (< 5)	63 (51.22)	1.24 ±1.09
Abnormal (≥ 5)	60 (48.78)	5.55 ±1.60
Homocysteine [µmol/l]:		10.34 ±7.71
Normal (< 12)	74 (77.89)	7.96 ±2.06
Abnormal (≥ 12)	21 (22.11)	18.79 ±13.08
CCA-IMT [mm]:		0.73 ±0.16
Normal (< 0.8)	71 (68.54)	0.649 ±0.094
Abnormal (≥ 0.8)	32 (31.46)	0.916 ±0.15
Waist circumference [cm]:		93.72 ±14.08
Male	61 (48.66)	95.53 ±12.74
Female	46 (27.98)	91.47 ±15.58
Physical activity:		
No	20 (15.50)	
Yes	109 (84.50)	
Smoking:		
No	121 (93.80)	
Yes	8 (6.20)	
Type 2 diabetes (T2D):		
No	89 (69.53)	
Yes	39 (30.47)	
Hypertension[$]:		
No	70 (54.69)	
Yes	58 (45.31)	
Family history of T2D:		
No	48 (43.24)	
Yes	63 (56.76)	
Family history of CAD:		
No	67 (60.36)	
Yes	44 (39.64)	
MS[†]:		
No	68 (53.1)	
Yes	38 (29.7)	

[†]MS defined by International Diabetes Federation criteria; Dys-HDL – dysfunctional HDL measured by HDL inflammatory index; hsCRP – high-sensitivity C reactive protein, CCA-IMT – common carotid artery intima media thickness; [$]history/examination and/or blood test

On Fisher's exact test analysis, presence of both MS (p = 0.0348) and Dys-HDL was significantly correlated with CCA-IMT, even after age and gender adjustment (Table III). The odds of having Dys-HDL with CCA-IMT were 5 times (95% confidence interval (95% CI) 1.68, 10.78) than with those without Dys-HDL (Table IV).

Discussion

Study findings presented here indicate Dys-HDL as a possible risk factor for subclinical atherosclerosis on carotid ultrasound, manifested as high CCA-IMT. To the best of our knowledge, thus is the first study showing the association of Dys-HDL with CCA-IMT. These results further confirm

Table III. Association of CCA-IMT (age and gender adjusted) with MS and Dys-HDL (n = 129)

Variable		CCA-IMT		Value of $p^{\$}$
		< 0.8 mm	≥ 0.8 mm	
MS†	0	46 (55.42)	15 (18.07)	0.0348
	1	11 (13.25)	11 (13.25)	
Dys-HDL	HDL index < 1	48 (51.61)	16 (17.20)	0.0024
	HDL index ≥ 1	12 (12.90)	17 (18.28)	

CCA-IMT – common carotid artery intima media thickness; †MS – metabolic syndrome defined according to International Diabetes Federation criteria [Central obesity and two or more of fasting glucose (≥ 100 mg/dl or T2D), obesity, HDL (≥ 40 mg/dl), blood pressure (≥ 130/≥ 85 mm Hg); and triglycerides (≥ 150 mg/day)]; Dys-HDL – dysfunctional HDL measured by HDL inflammatory index; $Fisher's exact test

the findings of our previous study on Dys-HDL and its correlation with increased CCA-IMT in a small cohort of SAIs in the US, even after adjusting for quantitative HDL level, age, family history of CAD, and hypertension [19]. Normal HDL possesses anti-atherogenic properties. HDL transports excess cholesterol from cells in artery walls to the liver for disposal [16, 28], removes reactive oxygen species from oxidized (Ox)LDL, prevents OxLDL-mediated recruitment of inflammatory mediators and monocytes into the vessel wall and inhibits endothelial cell expression of adhesion molecules and release of hemokines/cytokines [29, 30]. Several components of HDL contribute to these protective effects, including apoA-1 and the enzyme paraoxonase [30]. Conversely, Dys-HDL cannot prevent oxidation of LDL and may even further enhance it, leading to impairment of reverse cholesterol transport, increased recruitment of monocytes, and probably an enhanced inflammatory response [28]. Multiple mechanisms confer pro-inflammatory characteristics on HDL molecules [31–33]. In acute inflammation, hepatic synthesis of the protective lipoproteins in HDL, including apoA-1 and antioxidant enzymes such as PON1, decreases [29]. Additionally, protective components in the HDL particles, such as apoA-1, are partly replaced with pro-oxidant acute phase reactants [17, 28]. Furthermore, HDL and apoA-1 can be readily oxidized during periods of inflammation by myeloperoxidase, a product of white blood cell activation [30]; oxidation of HDL probably contributes to its dysfunction.

CCA-IMT has been shown to be independently associated with CAD in South Asians [34] and is a reproducible clinical tool to evaluate atherosclerosis, predict CAD and show the effectiveness of medical therapies [6, 34, 35]. However, in this small study we have shown that CCA-IMT can also predict functionality of HDL and can be instrumental in assessing CAD risk, especially in those without CAD. In one of the studies, it was found that IMT is inversely associated with HDL levels in middle aged men, but directly associated in middle aged women, as anti-atherogenic effects of HDL diminish in women around the age of menopause [36]. However, in this study we observed an association of CCA-IMT with Dys-HDL in both men and women (not shown in the tables). In addition, CCA-IMT was also associated with biomarkers of CAD, including hsCRP and homocysteine, as well as risk factors of CAD, i.e. diabetes and hypertension (Table V).

Table IV. Association of CCA-IMT (as a categorical variable < 0.8 mm and ≥ 0.8 mm) with MS and Dys-HDL (n = 129)

Variable	OR (95% CI)	Wald X^2	Value of $p^{\$}$
MS	3.07 (1.11, 8.49)	3.42	0.0311
Dys-HDL	4.25 (1.68, 10.78)	9.28	0.0023

CCA-IMT – common carotid artery intima media thickness; MS – metabolic syndrome defined according to International Diabetes Federation criteria (central obesity and two or more of fasting glucose (≥ 100 mg/dl or T2D), obesity, HDL (≥ 40 mg/dl), blood pressure (≥ 130/≥ 85 mm Hg); and triglycerides (≥ 150 mg/day)); Dys-HDL – dysfunctional HDL measured by HDL inflammatory index; OR – odds ratio; CI – confidence interval; $logistic regression

Elevated homocysteine levels have attracted much attention in recent years as a potential risk factor for CVD [37, 38]. Also, higher homocysteine levels have been linked to carotid atherosclerosis as assessed by IMT [39]. The homocysteine levels may be determined by genetic and/or environmental factors, among which low dietary folate and vitamin B_6 intake are important for inducing hyperhomocysteinemia [40]. Compared with North America and Europe, vegetable consumption is relatively high and meat consumption is relatively low in South Asian Hindus (South Asians belonging to the Hindu religion), implying more intake of folate and vitamin B_6. Previous research on homocysteine in South Asians is not conclusive. Many studies have not found any significant association between the two [41, 42]. To our knowledge, this is the first study in SAIs showing an association of homocysteine with CCA-IMT. However, the results must be interpreted with caution because of the small numbers studied.

Regarding MS, there is much controversy and contention associated with the term. Metabolic syndrome as a concept was first introduced in Sweden in 1923, to define the association of hy-

Table V. Association of CCA-IMT (age and gender adjusted) with coronary artery disease (CAD) risk factors (n = 129)

Variable		IMT		Value of p*
		0	1	
Age [years]	> 40	10 (8.09)	2 (1.83)	0.203
	≤ 40	64 (62.88)	32 (31.19)	
Gender	Male	44 (37.61)	20 (19.27)	0.557
	Female	30 (29.36)	14 (13.76)	
Smoke	No	70 (63.3)	32 (31.19)	1.000
	Yes	4 (3.67)	2 (1.83)	
Physical activity	No	9 (8.26)	8 (7.34)	0.112
	Yes	65 (58.72)	26 (25.69)	
Type II diabetes (T2D)	No	56 (53.21)	17 (14.68)	0.008
	Yes	18 (13.76)	17 (14.68)	
Hypertension†	No	47 (41.28)	13 (11.93)	0.012
	Yes	27 (25.69)	21 (21.1)	
Family history of T2D	No	28 (29)	14 (14)	0.512
	Yes	37 (37)	17 (20)	
Family history of CAD	No	40 (50)	17 (19)	0.342
	Yes	25 (16)	14 (15)	
Waist circumference [cm]	< 90 (Male), < 80 (Female)	21 (22.99)	3 (3.45)	0.105
	≥ 90 (Male), ≥ 80 (Female)	45 (50.57)	18 (22.99)	
Total HDL [mg/dl]	Normal (> 40)	49 (54.72)	19 (22.64)	0.136
	Abnormal (≤ 40)	22 (12.26)	15 (10.38)	
Total LDL [mg/dl]	Normal (< 130)	57 (55.24)	30 (29.52)	0.139
	Abnormal (≥ 130)	14 (12.38)	3 (2.86)	
Triglycerides [mg/dl]	Normal (< 150)	42 (38.89)	20 (20.37)	0.565
	Abnormal (≥ 150)	29 (27.78)	14 (12.96)	
Total cholesterol [mg/dl]	Normal (< 200)	41 (34.91)	19 (18.87)	0.511
	Abnormal (≥ 200)	30 (32.08)	15 (14.15)	
Apo lipoprotein A-1	Normal (94–176)	56 (52.83)	23 (21.7)	0.089
	Abnormal	13 (14.15)	12 (11.32)	
Lipoprotein [a] [mg/dl]	Normal (< 10)	46 (44.76)	16 (16.19)	0.087
	Abnormal (≥ 10)	25 (22.86)	17 (16.19)	
hsCRP [mg/l]	Normal (< 5)	42 (40.2)	6 (6.86)	< 0.001
	Abnormal (≥ 5)	29 (27.45)	26 (25.49)	
Homocysteine [µmol/l]	Normal (< 12)	42 (53.25)	20 (25.97)	0.051
	Abnormal (≥ 12)	13 (18.18)	1 (2.6)	

*n (%); hsCRP – high-sensitivity C reactive protein; †History/examination;*Fisher's exact test*

perglycemia, hypertension and gout [43]. The majority of SAIs (preferably South Asian Indians) have a smaller body habitus compared to Caucasians. Obesity, by its classic definition, is rare among SAIs [44], as seen in this study as well (Tables II and V). The new International Diabetes Federation (IDF) definition attempted to compensate (to a very limited extent) for this flaw by recognizing a different set of measurements to define abdominal obesity in different Asian ethnic groups from the different regions in the Asian continent [45]. It is widely recognized that there are variations in the combinations of different metabolic derangements in different populations groups, which can lead to a wide array of disease outcomes, of which the majority are cardiovascular in nature. Thus, it is clear that the traditional definitions of MS still fail to be representative of South Asian Indian ethnicity. It is interesting to note that the conventional descriptions of MS have no mention of genetic factors that serve as CAD risk markers. In defining the MS unique to the Asian Indian, it is important to recognize the accelerated progression of coronary atherosclerosis, leading to myocardial

infarction at a younger age. The unique constellation of known metabolic risk factors that predisposes the SAIs to CAD and its manifestation early in life can be identified early in life. The distinct features of this MS with accelerated atherosclerosis in the SAIs include visceral adiposity, insulin resistance/hyperinsulinemia, high LP(a) levels, low HDL levels, and high homocysteine levels. The current study also confirmed the findings of an earlier study showing significant prevalence of MS in SAIs, including a high burden of CAD risk factors. In addition, MS was significantly associated with CCA-IMT. Many risk factors are associated with carotid atherosclerosis, and several epidemiological studies have demonstrated that MS is an independent risk factor for carotid atherosclerosis [46–48]. Consistent with previous findings, the current study found that CCA-IMT was significantly greater in subjects with MS than in those without. Furthermore, the results of our study are consistent with those of previous studies that have found a trend toward increased CCA-IMT with increasing numbers of components of MS (Table V). Kanaya *et al.* also found an association of CCA-IMT with MS in SAIs in a study that was designed based on MESA (Multi-Ethnic Study of Atherosclerosis) study methods [49].

First, the study design was cross-sectional, so caution should be exercised in any causal interpretation of CCA-IMT and the CAD parameters. Additionally, longitudinal effects of Dys-HDL, MS and other CAD risk factors on the progression of CCA-IMT were not evaluated. A second limitation of our study is the small sample size due to limited funding. Further longitudinal studies are needed to support findings in the larger SAI population. Third, the convenience sample of the study may cause selection bias. We recruited participants from local Hindu temples, and therefore participants may not be completely representative of the South Asian Indian community. There are no census data on SAIs within the US that provide a true estimate of the SAI population, and the convenience sampling method is mostly used to design studies in SAIs. However, people attending these temples were from mixed ethnic backgrounds, and data were collected from participants who attended weekend worship services, which in general are attended by SAI Hindus from different and diverse ethnic groups. Therefore, we anticipate that the selection bias is minimal and that the sample is representative of SAI Hindus. Third, we did not assess diet in this study, and we plan to include a detailed nutrition component in our larger study. Fourth, due to the limited budget and in order to have a homogeneous group, we only included Hindu SAIs, and SAs of different religions were not included. And last but not the least, we were not able to do further special tests, and, since this was a small sample size, we were not able to make any definitive conclusions regarding the association of Dys-HDL with CAD and its risk factors. Although this study used the presence of CCA-IMT as an outcome variable, more precise analyses must use characteristics of plaques (volume, height, and echogenicity) rather than merely the presence of CCA-IMT as an outcome.

In conclusion, this study is the first of its kind assessing the association of sub-clinical CAD using carotid IMT as a surrogate marker of atherosclerosis with Dys-HDL in SAIs. Given that SAIs are known to carry a disproportionately high risk for CAD, there is a need to explore and understand non-traditional risk factors. The SAs in general and SAIs in particular are known to have low HDL. This study has also highlighted the presence of Dys-HDL in this high risk group. Therefore, not only HDL quantity but also HDL quality and function are important for CAD future prediction. A major challenge associated with primary prevention of CAD in SAIs involves the early and accurate detection of CAD in high-risk but asymptomatic individuals, to prevent coronary events. CCA-IMT is a non-invasive surrogate marker of atherosclerosis and is proven to be helpful in detecting sub-clinical CAD by stratifying populations at highest risk for CAD. The results of this study further suggest that health education and rehabilitation programs need to be designed specifically for this high-risk group, would be beneficial when initiated early in life, and need to be targeted at the individual. Although the major modifiable risk factors do not fully explain the excess burden of CAD, they are doubly important and remain the foundation of preventive and therapeutic strategies in this population. A more aggressive approach to preventive therapy, especially dyslipidemia, at an earlier age and at a lower threshold, is clearly warranted. In addition, determining the presence of Dys-HDL in SAIs will answer several questions related to the presence of altered HDL level and function. Further research, including longitudinal prospective studies, is required to support the current study findings with investigation of the temporal association.

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References

1. Wilson PWF, Castelli WP, Kannel WB. Coronary risk prediction in adults: the Framingham Heart Study. Am J Cardiol 1987; 59: 91-4.
2. Smith SC Jr, Greenland P, Grundy SM. Prevention conference V: beyond secondary prevention: identifying the high-risk patient for primary prevention. Circulation 2000; 101: 111-6.
3. Lloyd-Jones D, Adams R, Carnethon M, et al. Heart disease and stroke statistics – 2009 update: a report from the American heart association statistics committee and stroke statistics subcommittee. Circulation 2009; 119: 480-6.
4. ACAPS Group. Rationale and design for the Asymptomatic Carotid Artery Plaque Study. Control Clin Trials 1992; 13: 293-314.
5. Rosa EM, Kramer C, Castro I. Association between coronary artery atherosclerosis and the intima-media thickness of the common carotid artery measured on ultrasonography. Arq Bras Cardiol 2003; 80: 589-92, 285-8.
6. Del Sol AI, Moons KM, Hollander M, et al. Is carotid intima-media thickness useful in cardiovascular disease risk assessment? The Rotterdam Study. Stroke 2001; 32: 1532-8.
7. Uppaluri CR. Heart disease and its related risk factors in Asian Indians. Ethn Dis 2002; 12: 45-53.
8. Enas EA, Senthilkumar A. Coronary artery disease in Asian Indians: an update and review. Internet J Cardiol 2001; 1: 2.
9. Bedi US, Singh S, Syed A, Aryafar H, Arora R. Coronary artery disease in South Asians: an emerging risk group. Cardiol Rev 2006; 14: 74-80.
10. Yusuf S, Hawken S, Ounpuu S, et al.; INTERHEART Study Investigators. Effects of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 2004; 364: 937-52.
11. Goyal A, Yusuf S. The burden of cardiovascular disease in the Indian subcontinent. Indian J Med Res 2006; 124: 235-44.
12. Forouhi NG, Sattar N, Tillin T, McKeigue PM, Chaturvedi N. Do known risk factors explain the higher coronary heart disease mortality in South Asian compared with European men? Prospective follow-up of the Southall and Brent studies, UK. Diabetologia 2006; 49: 2580-8.
13. Joshi P, Islam S, Pais P, et al. Risk factors for early myocardial infarction in South Asians compared with individuals in other countries. JAMA 2007; 297: 286-94.
14. Gordon DJ, Probstfield JL, Garrison RJ, et al. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. Circulation 1989; 79: 8-15.
15. Navab M, Anantharamaiah GM, Reddy ST, Van Lenten BJ, Ansell BJ, Fogelman AM. Mechanisms of disease: proatherogenic HDL – an evolving field. Nat Clin Pract Endocrinol Metab 2006; 2: 504-11.
16. Navab M, Hama SY, Anantharamaiah GM, et al. Normal high density lipoprotein inhibits three steps in the formation of mildly oxidized low density lipoprotein: steps 2 and 3. J Lipid Res 2000; 41: 1495-508.
17. Ansell BJ, Watson KE, Fogelman AM, Navab M, Fonarow GC. High-density lipoprotein function recent advances. J Am Coll Cardiol 2005; 46: 1792-8.
18. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001; 285: 2486-97.
19. Dodani S, Kaur R, Reddy S, Reed GL, Mohammad N. Can dysfunctional HDL explain high coronary artery disease risk in South Asians? Int J Cardiol 2008; 129: 125-32.
20. Pignoli P, Tremoli E, Poli A, Oreste P, Paoletti R. Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. Circulation 1986; 77: 1399-406.
21. O'Leary DH, Polak JF, Wolfson SK Jr, et al. Use of sonography to evaluate carotid atherosclerosis in the elderly. The Cardiovascular Health Study. CHS Collaborative Research Group. Stroke 1991; 22: 1155-63.
22. Touboul PJ, Hennerici MG, Meairs S, et al. Mannheim carotid intima-media thickness consensus (2004-2006). An update on behalf of the advisory board of the 3rd and 4th watching the risk symposium 13th and 15th European stroke conferences, Mannheim, Germany, 2004, and Brussels, Belgium, 2006. Cerebrovasc Dis 2007; 23: 75-89.
23. Howard G, Manolio TA, Burke GL, Wolfson SK, O'Leary DH. Does the association of risk factors and atherosclerosis change with age? An analysis of the combined ARIC and CHS cohorts. The Atherosclerosis Risk in Communities (ARIC) and Cardiovascular Health Study (CHS) investigators. Stroke 1997; 28: 1693-701.
24. Ansell BJ, Fonarow GC, Fogelman AM. High-density lipoprotein: is it always atheroprotective? Curr Atheroscler Rep 2006; 8: 405-11.
25. Navab M, Hama SY, Hough GP, Subbanagounder G, Reddy ST, Fogelman AM. A cell-free assay for detecting HDL that is dysfunctional in preventing the formation of or inactivating oxidized phospholipids. J Lipid Res 2001; 42: 1308-17.
26. Ansell BJ, Navab N, Hama S, et al. Inflammatory/antiinflammatory properties of high-density lipoprotein distinguish patients from control subjects better than high-density lipoprotein cholesterol levels and are favorably affected by Simvastatin treatment. Circulation 2003; 108: 2751-6.
27. Dodani S, Kaur R, Reed GL, George V, Sharma G, Marion L. The association of carotid intima media thickness with dysfunctional HDL in South Asians. Arch Med Sci 2008; 4: 40-6.
28. Navab M, Berliner JA, Watson AD, et al. The Yin and Yang of oxidation in the development of the fatty streak. A review based on the 1994 George Lyman Duff Memorial Lecture. Arterioscler Thromb Vasc Biol 1996; 16: 831-42.
29. Cockerill GW, Rye KA, Gamble JR, Vadas MA, Barter PJ. High-density lipoproteins inhibit cytokine induced expression of endothelial cell adhesion molecules. Arterioscler Thromb Vasc Biol 1995; 15: 1987-94.
30. Zhang Y, Zanotti I, Reilly MP, Glick JM, Rothblat GH, Rader DJ. Overexpression of apolipoprotein A-I promotes reverse transport of cholesterol from macrophages to feces in vivo. Circulation 2003; 108: 661-3.
31. Barter PJ, Baker PW, Rye KA. Effect of high-density lipoproteins on the expression of adhesion molecules in endothelial cells. Curr Opin Lipidol 2002; 13: 285-8.
32. Ferretti G, Bacchetti T, Negre-Salvayre A, Salvayre R, Dousset N, Curatola G. Structural modifications of HDL and functional consequences. Atherosclerosis 2006; 184: 1-7.

33. Nicholls SJ, Zheng L, Hazen SL. Formation of dysfunctional high-density lipoprotein by myeloperoxidase. Trends Cardiovasc Med 2005; 15: 212-9.
34. Hodis HN, Mack WJ, LaBree L, et al. The role of carotid arterial intima media thickness in predicting clinical coronary events. Ann Intern Med 1998; 128: 262-9.
35. Jadhav UM, Kadam NN. Carotid Intima-media thickness as an independent predictor of coronary artery disease. Indian Heart J 2001; 53: 458-62.
36. Miller GJ, Miller NE. Plasma-high-density-lipoprotein concentration and development of ischaemic heart disease. Lancet 1975; 1: 16-9.
37. Stampfer MJ, Malinow MR, Willett WC, et al. A prospective study of plasma homocysteine and risk of myocardial infarction in US physicians. JAMA 1992; 268: 877-81.
38. Clarke R, Daly L, Robinson K, Naughten E, Cahalane S, Fowler B. Hyperhomocysteinemia: an independent risk factor for vascular disease. N Engl J Med 1991; 324: 1149-55.
39. Selhub J, Jacques PF, Bostom AG, et al. Association between plasma homocysteine concentrations and extracranial carotid-artery stenosis. N Engl J Med 1995; 332: 286-91.
40. Selhub J, Jacques PF, Wilson PW, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. JAMA 1993; 270: 2693-8.
41. Deepa R, Velmurugan K, Saravanan G, Karkuzhali K, Dwarakanath V, Mohan V. Absence of association between serum homocysteine levels and coronary artery disease in south Indian males. Indian Heart J 2001; 53: 44-7.
42. Sastry BK, Indira N, Anand B, Kedarnath, Prabha BS, Raju BS. A case-control study of plasma homocysteine levels in South Indians with and without coronary artery disease. Indian Heart J 2001; 53: 749-53.
43. Kylin E. Studies of the hypertension-hyperglycaemia-hyperuricaemia syndrome. Zentralbl Inn Med Leipzig 1923; 44: 105-27.
44. Deepa M, Farooq S, Datta M, Deepa R, Mohan V. Prevalence of metabolic syndrome using WHO, ATPIII and IDF definitions in Asian Indians: the Chennai Urban Rural Epidemiology Study (CURES-34). Diabetes Metab Res Rev 2007; 23: 127-34.
45. Gonzalez-Ortiz M, Martinez-Abundis E, Jacques-Camarena O, Hernandez-Gonzalez SO, Valera-Gonzalez IG, Ramos-Zavala MG. Prevalence of metabolic syndrome in adults with excess of adiposity: comparison of the Adult Treatment Panel III criteria with the International Diabetes Federation definition. Acta Diabetol 2006; 43: 84-6.
46. Tzou WS, Douglas PS, Srinivasan SR, et al. Increased subclinical atherosclerosis in young adults with metabolic syndrome: The Bogalusa Heart Study. J Am Coll Cardiol 2005; 46: 457-63.
47. McNeill AM, Rosamond WD, Girman CJ, et al. Prevalence of coronary heart disease and carotid arterial thickening in patients with the metabolic syndrome (The ARIC Study). Am J Cardiol 2004; 94: 1249-54.
48. Pollex RL, Al-Shali KZ, House AA, et al. Relationship of the metabolic syndrome to carotid ultrasound traits. Cardiovasc Ultrasound 2006; 4: 28.
49. Kanaya AM, Wassel CL, Mathur D, et al. Prevalence and correlates of diabetes in South Asian indians in the United States: findings from the metabolic syndrome and atherosclerosis in South Asians living in america study and the multi-ethnic study of atherosclerosis. Metab Syndr Relat Disord 2010; 8: 157-64.