The association of carotid intima media thickness with dysfunctional HDL in South Asians

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Abstract

Introduction: Coronary artery disease (CAD) is the leading cause of death in the United States and its rates are increasing in South Asians. Screening and detection of CAD risk factors can help prevent future risk CAD by early treatment. The objective of the current study was to determine the association of sub-clinical CAD using carotid intima media thickness (IMT) as a surrogate marker of atherosclerosis and dysfunctional HDL assess by novel cell free assay test in South Asians.

Material and methods: A small pilot study on South Asians between the ages of 40-65 years was conducted. Carotid IMT was performed and dysfunctional HDL was assessed by novel cell-free assay.

Results: The prevalence of sub-clinical CAD using carotid IMT (≥0.80 mm) as a surrogate marker for atherosclerosis was seen in 41.4% [95% confidence limits (CL), 0.2347-0.5933]. HDL inflammatory Index values of ≥1.00 were seen in 14 subjects (50%), suggesting pro-inflammatory (dysfunctional) HDL with (95% CL, 0.8772-1.4333). On logistic regression analysis, IMT values ≥0.80 mm was associated with HDL inflammatory index (p=0.02), even after adjusting for age (p=0.03).

Conclusions: The results of this study can be useful to explore further the associations and causes of dysfunctional HDL in South Asians in larger prospective studies. Emphasis needs to be given not only to the HDL levels but also the functionality of HDL, especially those with CAD risk factors who are at higher risk of future CAD. Early detection and treatment of dysfunctional HDL may reduce future CAD risk.

Key words: South Asians, dysfunctional HDL, intima-media thickness, coronary artery disease.

Introduction

Among cardiovascular diseases, coronary artery disease (CAD) is the leading cause of mortality and morbidity in the United States (US) [1, 2]. Even though CAD event rates have been 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 been doubled in the past two decades [2] South Asians, especially South Asian immigrants (SAIs) exhibit the higher prevalence of CAD and coronary risk factors as compare with Caucasians (10 vs. 2.5%) [3, 4] and these findings are not limited to the US but appears to be a global phenomenon [5].

Although South Asians represent the second fastest growing Asian immigrant population in the US, little is known regarding their increased risk for CAD [2]. Not a uniform group, SAIs includes ethnic subgroups with different cultures and practices, and the prevalence of recognized risk factors for CAD varies among the subgroups. As a whole, however, SAIs, compared to other populations, have much higher prevalence of diabetes, metabolic syndrome, insulin resistance, central obesity, dyslipidemias [lower HDL, increased lipoprotein a (Lp[a]), higher triglycerides (TGs), increased thrombotic tendency, and low levels of physical activity] [6-10]. Further, it has been seen that 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 [11]. However, even taking these differences into account, conventional risk factors, insulin resistance parameters, or metabolic syndrome, although important in predicting CAD risk, may not fully account for the increased risk in SAIs [9, 10]; thus, a search for additional markers is warranted, to promote early detection and prevention of CAD in this high risk group.

Knowing the high prevalence of CAD and its risk factors in South Asian, a major challenge associated with primary prevention of CAD involves early risk factors detection as well as early diagnosis of sub-clinical CAD in those individuals who are at risk but are asymptomatic. Sub-clinical CAD is known to be a significant predictor of subsequent cardiac events [12, 13]. Early detection of sub-clinical CAD in high risk South Asian at a young age could help prevent coronary events and substantially reduce the level of death and disability attributable to CAD. Non-invasive surrogate markers of atherosclerosis, such as common carotid artery intima media thickness (IMT), have been found to be helpful in detecting sub-clinical CAD by identifying those at high risk of coronary events [13-16]. Increased carotid IMT measurements have been employed to predict the extent and severity of CAD [17-19] and have been found to be strongly associated with an increased risk of cardiovascular morbidity and mortality [19-21], as well as a marker of atherosclerosis regression and dyslipidemias improvement in patients on lipid lowering therapy [22, 23].

Pro-inflammatory (dysfunctional) HDL

Among numerous genetic and lifestyle parameters, dyslipidemias are one of the most prominent risk factors for CAD. High density lipoprotein (HDL) cholesterol plays a protective role in preventing CAD and low HDL is an independent risk factor for CAD [24, 25]. This protective effect of HDL is related to its role as an anti-atherogenic agent that prevents low density lipoprotein (LDL) oxidation. According to several recent Caucasian studies, in patients with CAD, HDL is not only *ineffective* as an antioxidant but, paradoxically, appears to be pro-oxidant, as assessed by its lipid peroxide content [26-30]. This pro-inflammatory HDL, named as dysfunctional HDL (Dys-HDL), accumulates oxidants that inhibit HDL-associated antioxidant enzymes, render Apolipoprotein A-I (Apo A-I), 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 [31]. Current data indicates that a 1% increase in HDL serum concentration can decrease cardiovascular risk by 2-3%, independent of LDL levels [23]. However, HDL can have this protective effect only if it is functional. The prevalence of pro-oxidant or pro-inflammatory HDL (dysfunctional HDL) in South Asian is not yet known.

The objective of the current study was to determine the association of sub-clinical CAD measured by carotid IMT as a surrogate marker of atherosclerosis in South Asians with dysfunctional HDL. In this paper, dysfunctional HDL, pro-inflammatory and HDL inflammatory index are used interchangeably.

Material and methods

In this cross-sectional pilot study, SAIs between the ages of 40-65 years were recruited from the main Hindu temples in Georgia State. Written informed consent and ethics approval was obtained from study subjects and university institutional review board respectively. Information on socio-demographics, personal lifestyle characteristics, and CAD risk factors (both traditional and specific) was gathered (Table I and Table II). Twelve hour fasting blood samples were collected for measurements of C-reactive protein, total cholesterol, triglycerides (TGs), HDL, LDL, lipoprotein a (Lpa) and insulin levels.

Carotid ultrasound Doppler

B-mode ultrasound scanning of bilateral carotid arteries was performed by a trained non-invasive vascular ultrasound technician at both the sites using SonoCalc™ IMT machine (SonoSite, Inc Bothell, WA) with a 10.0 MHz linear array transducer. Both common carotid arteries were scanned in supine position. A total of four images will be obtained on each side, 1 cm proximal to the carotid bulb using an anterior approach. ECG leads was placed to obtain end-diastolic measurements. Images were recorded and stored on a disk. The common carotid artery approach for IMT

Table I. Demographic characteristics of study group (N=29)

Variable	n (%)
Age	56* (6.47†)
Gender	
male	14 (48.3)
female	15 (51.7)
Ethnicity	
Gujarati	6 (20.7)
Hindi	15 (51.7)
South Indian	5 (17.2)
Bengali	1 (3.4)
Punjabi	1 (3.4)
Marathi	1 (3.4)
Work type	
medical doctor	2 (6.9)
business	3 (10.3)
government job	8 (27.6)
engineer	5 (17.2)
housewife	4 (13.8)
others	7 (24.1)
Education	
undergraduate	7 (24.1)
graduate	9 (31.0)
postgraduate	13 (44.8)

**Mean, †standard deviation*

measurements was preferred because the common carotid IMT is reproducible and predictive of future cardiovascular events, and the data collection is more complete than other non-invasive markers [32-35]. Measurements of the internal carotid and bifurcation segments tend to have many more missing values [33, 35]. The Mannheim Intima-Media Thickness Consensus suggested that measurement of the common carotid is ideal [36].

IMT defined by Pignoli and colleagues 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 [37, 38]. Any focal thickening of the intima-media complex or carotid plaque was not included in the analysis. A cardiologist who was blinded to participants, clinical information, analyzed stored images by using automated edge detection technology (SonoCalc™ IMT). Measurement of the far wall of the carotid artery was preferred. Studies comparing ultrasound measurements with histology suggest that far-wall common carotid IMT measurements are more indicative of the true thickness of the arterial wall [38-40]. Near-wall common carotid

Table II. Sub-clinical CAD and risk factors in study group (N=29)

Variable	n/N (%)	Mean ± Std.
CAD		0.77±0.18
IMT ≥0.80	12/29 (41.4)	0.92±0.15
IMT <0.80	17/29 (58.6)	0.67±0.11
HDL inflammatory index		1.09±0.70
≥1.00	14/28 (50)	1.57±0.69
<1.00	14/28 (50)	0.60±0.12
Cholesterol		207.36±41.65
desirable (<200)	13/28 (44.8)	174.23±16.35
borderline high (200-239)	10/28 (34.5)	215.10±11.78
high (≥240)	5/28 (17.2)	278.00±25.31
HDL		51.29±8.74
low (<40)	4/28 (13.8)	37.75±1.89
normal (40-59)	18/28 (62.1)	50.50±5.31
high (≥60)	6/28 (20.6)	62.67±3.27
LDL		134.43±40.55
optimal (<100)	6/28 (20.7)	90.50±7.34
above optimal (100-129)	8/28 (27.6)	115.88±8.71
borderline high (130-159)	8/28 (27.6)	139.25±6.67
high (160-189)	3/28 (10.3)	169.00±3.00
very high (≥190)	3/28 (10.3)	224.33±21.50
Triglycerides		108.11±46.05
normal (<150)	23/28 (79.3)	90.52±25.63
borderline high (150-199)	3/28 (10.3)	170.33±10.41
high (200-249)	2/28 (6.9)	217.00±11.31
Lipoprotein(a)		31.07±28.90
normal (<39)	21/28 (72.4)	19.62±10.79
borderline high (39-49)	3/28 (10.3)	45.67±5.77
high (50-59)	3/28 (10.3)	56.00±4.36
very high (≥60)	1/28 (3.4)	153.00
BMI		24.93±2.46
normal (18.5-24.9)	17/29 (58.6)	23.22±1.44
pre-obese (25-29.9)	12/29 (41.4)	27.35±1.21
Waist circumference		90.97±15.60
males	14/29 (48.3)	95.14±19.85
≥102	1/14 (7.1)	162.00
<102	13/14 (92.9)	90.00±5.08
females	15/29 (51.7)	87.07±9.32
≥88	8/15 (53.3)	94.13±5.99
<88	7/15 (46.7)	79.00±4.28
Hypertension		
yes	5/29 (17.2)	
no	24/29 (82.8)	
Diabetes		
yes	2/29 (6.9)	
no	27/29 (93.1)	
Family history of CVD		
yes	14/29 (48.3)	
no	15/29 (51.7)	
Physical activity		
yes	26/29 (89.7)	
no	3/29 (10.34)	

BMI – body mass index, CVD – cardiovascular disease

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 [41].

Participants with values greater than 0.80 mm were considered to be reported as IMT positive. Previous epidemiological studies suggest that a value of intima-media thickness at or above 0.80 mm is associated with a significantly increased absolute risk of CAD [19, 20]. Carotid IMT values were adjusted for age as age can influence IMT readings [42, 43].

Dysfunctional HDL and inflammatory index

The diagnosis of dysfunctional 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 novel cell free assay has been developed to detect HDL that is dysfunctional [44]. 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 detailed cell free assay method will be provided once accepted.

Similarly, the HDL-inflammatory index (HII) was calculated by normalizing the cell free assay values obtained for LDL alone as <1.0 [45]. If 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.

Table III. IMT as a categorical variable, ≥0.8 vs. <0.8 with CAD risk factors

Predictors	Wald Chi-square	Odds ratio (95% CI)	p* value
Age group	0.0036	–	0.9522
Gender	0.3564	1.58 (0.35±7.00)	0.5505
Hypertension	0.8303	2.50 (0.35±17.94)	0.3622
Diabetes	0.0652	1.46 (0.08±25.81)	0.7985
Family history	2.6687	3.67 (0.77±17.43)	0.1023
Physical activity	0.8138	3.20 (0.26±40.06)	0.3670
BMI	0.0007	1.02 (0.23±4.57)	0.9789
HDL	0.0966	1.40 (0.17±11.68)	0.7559
Triglycerides	0.0203	0.87 (0.12±6.22)	0.8868
Lipoprotein(a)	0.7565	0.44 (0.07±2.80)	0.3844
LDL	0.2795	1.67 (0.25±11.07)	0.5970
C-reactive protein	0.0008	–	0.9779
HDL inflammatory index	4.8426	6.60 (1.23±35.44)	0.0278
Cholesterol	1.1777	0.43 (0.09±1.98)	0.2778

**Univariate logistic regression, IMT – intima media thickness, BMI – body mass index*

Data analysis

For this pilot study, we recruited a fixed sample of 30 South Asians, selected from the two main Hindu temples in the State of Georgia. These temples are attended by South Asians with different ethnic backgrounds.

Windows-based SAS software version 9.1 was used for all data management and statistical analyses. A detailed descriptive statistics were conducted to explore distribution of CAD risk factors, including demographics and other relevant variables. Univariate as well as multivariate logistic regression (using backward and forward model selection procedures) was done for associations of Carotid IMT with CAD risk factors.

Results

Of the total of 30 subjects, one could not complete the study questionnaire and blood work, and was therefore excluded from the study. We could not draw blood from one subject but IMT and other information were obtained and were included in the study. Therefore, a total of 29 subjects were included in the study for IMT and other information but only 28 subjects were included for blood work.

The mean age of subjects was 56±6.46 years with an almost equal number of males and females (Table I). Majority were Hindi speaking professionals. The prevalence of CAD risk factors was: (a) hypertension 17%, (b) diabetes 6.9%, (c) high cholesterol (≥200 mg/dl) 34.5%, (d) HDL 40 mg in 13.8%, and (e) positive family history of cardiovascular disease (CVD) in 48.3% (Table II). Furthermore, 41.45% were overweight and no one was a current smoker. The prevalence of sub-clinical CAD using carotid IMT (≥0.80 mm) as a surrogate marker for atherosclerosis was seen in 41.4% (95% CL 0.2347-0.5933) as shown in Table II. Using the normalization method, HDL inflammatory Index values of ≥1.00 were seen in 14 subjects (50%), suggesting pro-inflammatory (dysfunctional) HDL with (95% CL, 0.8772-1.4333).

Carotid IMT was significantly correlated with HDL inflammatory levels (Table III). On univariate as well multivariate logistic regression analysis (Table III and Table IV), IMT values ≥0.80 mm was associated with HDL inflammatory index (p=0.02). In addition, positive carotid IMT values remained significant with HDL inflammatory index after adjusting for age (p=0.03).

Discussion

To the best of our knowledge, this is the first study conducted in SAIs assessing the association of sub-clinical CAD, using carotid IMT as surrogate markers of atherosclerosis with dysfunctional HDL using novel test of cell free assay.

Carotid IMT has been shown to be independently associated with CAD in South Asians [46] and is a reproducible clinical tool to evaluate atherosclerosis, predict coronary artery disease and show the effectiveness of medical therapies [32, 34, 35, 39]. However, in this small study we have shown that carotid IMT can also predict functionality of HDL and can be instrumental in assessing CAD risk, especially in those without CAD. In one of the study it has shown 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 [47]. However, in this study we have seen strong association of IMT with dysfunctional HDL both with men and women (Table IV). We could not find the association of other CAD risk factors with IMT that could be because of the small sample size.

As shown in Table II, 12 (42.9%) of South Asians immigrants with ≥40 mg of HDL had dysfunctional HDL, in comparison to 2 (7.1%) with ≤40 mg/dl. Thus, from the HDL point of view, these subjects would not have been predicted to be at risk for atherosclerosis by conventional risk factor analysis.

Previous studies have shown low HDL levels associated with thickening of carotid IMT independent of other risk factors [48, 49]. However, we were not able to show this association and could be attributed to small sample size. Moreover, our study has shown association of IMT with dysfunctional HDL even after adjusting for age (Table IV).

Presence of dysfunctional/pro-inflammatory HDL can play a major role in predicting and monitoring atherosclerosis and it has been shown that Statins can favorably moderate the characteristics of proinflammatory HDL [50]. We believe that the results from this small group of South Asians immigrants will be found helpful to identify dysfunctional HDL and carotid IMT thickness in asymptomatic populations and could help in the prevention of future CAD by early treatment.

Several limitations of this study must be considered. First, this is a cross-sectional pilot study and, as in all such studies, the data are exploratory and do not allow the establishment of causality and do not account for changes over time. Second, we recruited participants from local Hindu temples and therefore, participants may not be completely representative of the South Asian community. However, people attending these temples were from mixed ethnic backgrounds, and data was collected from participants who attended weekend worship services, which in general are attended by South Asians from different and diverse ethnic groups. Therefore, we anticipate the selection bias is minimal, however it may not be representative of SAIs as we only considered Hindus in this study. We do plan to compare data with Caucasians in our larger study proposal submitted to the National Institute of Health (NIH).

Table IV. Intima media thickness (IMT) association with CAD risk factors (Outcome – IMT as a categorical variable, ≥0.8 vs. <0.8)

Variable	Wald Chi-square	Odds ratio (95% CI)	Value p*
HDL index (<1.0 vs. ≥1.0)	4.84	6.60 (1.23, 35.44)	0.0278
HDL index (<1.0 vs. ≥1.0)†	4.64	6.65 (1.19, 37.23)	0.0312

**Multivariate logistic regression, †adjusted for age*

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 dysfunctional HDL in SAIs. Given that SAIs are known to carry a disproportionately high risk for CAD, there is need to explore and understand non-traditional risk factors. A major challenge associated with primary prevention of CAD in South Asian involves the early and accurate detection of CAD in high risk, but asymptomatic individuals, to prevent coronary events. Common carotid IMT is a non-invasive surrogate marker of atherosclerosis and proven to be helpful in detecting sub-clinical CAD by stratifying populations at highest risk for coronary artery disease. In addition, determining the presence of dysfunctional HDL in South Asians will answer several questions related to the presence of altered HDL level and function. This information will not only help to stratify this high risk asymptomatic group, but will also be useful from a disease management point of view.

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Human Participants' Protection

This study was approved by the institutional review board and human subject committee at the Medical Collage of Georgia, Augusta GA.

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