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Research Article

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Association of Carotid Intima Media Thickness and Periscope Markers with Coronary Artery Disease, Risk factors and Biomarkers in Asian Indians

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Abstract

Surrogate sub clinical markers provide an important dimension to cardiovascular risk stratification. We studied the association of carotid intima media thickness (CIMT) and Periscope markers with CAD, classical risk factors and cardiac biomarkers in a cohort of 125 CAD patients with strong family history of cardiovascular disease and 585 unaffected family members selected from the Indian Atherosclerosis Research Study. CIMT was measured by B-mode ultrasound in 380 subjects while pulse wave velocity (PWV), arterial stiffness index (ASI) and ankle brachial index (ABI) was measured by Periscope in 710 subjects. CIMT, PWV and ASI were higher in affected than unaffected subjects (P<0.001). Mean IMT showed 20-fold and 6-fold higher risk for CAD before and after adjustment for age and gender, respectively, when the top and bottom tertile were compared (P<0.001), while unadjusted PWV showed 7-fold higher CAD risk. CIMT and Periscope markers were higher in the presence of classic cardiovascular risk factors, with linear association across Framingham risk categories. CIMT, PWV, ASI correlated well with waist circumference and age (r 0.16-0.50, p<0.001). Levels of total cholesterol, triglycerides, low-density-lipoprotein cholesterol and factor VII.c were elevated across the higher tertile of IMT, PWV and ASI (P ≤ 0.03). Mean CIMT showed an increase in the presence of plaque and was lower in CAD patients on statins. Risk prediction model that combined the classical risk factors, IMT and Periscope markers yielded the highest c index (AUC 0.906, 95% CI 0.880-0.932), with 34% reclassification in the intermediate risk group. In conclusion, both CIMT and Periscope markers show significant association with CAD and its established risk factors, suggesting that they can provide incremental value for early prediction of CAD in Asian Indians.

Keywords

Carotid intima media thickness; Periscope; Coronary artery disease; Biomarkers; Asian Indians

Introduction

Endothelial dysfunction is an early, potentially reversible process in the development of coronary artery disease (CAD). Timely detection of sub clinical changes in the endothelium can help in taking proactive measures against subsequent progression into overt cardiovascular events. In this regard, Carotid Intima Media Thickness (CIMT) and markers of vascular dysfunction in peripheral circulation, namely Pulse Wave Velocity (PWV), Arterial Stiffness Index (ASI) and Ankle Brachial Index (ABI) have demonstrated independent ability to predict risk of cardiovascular events including CAD and stroke [1-4]. Based on the strength of evidence obtained from clinical studies, both CIMT and ABI have been included in the recent guidelines drawn by the working committee of American Heart Association for assessing cardiovascular risk in asymptomatic adults [5]. Furthermore, these clinical indices are also associated with conventional cardiometabolic risk factors such as age, gender, hypertension [6] diabetes and metabolic syndrome [7].

From a perspective of timely prevention and effective disease management, a true understanding of the predictive utility of these clinical markers gains utmost importance for stratifying the high risk population. Being safe, easy to measure and reproducible, these techniques are most suitable for seamless integration in a clinical setting. Limited studies on Asian Indians have shown the association of both CIMT and Periscope markers with CAD and conventional risk factors [4,8,9]. This pilot study has been designed to assess the independent as well as combined value of CIMT and Periscope markers in discriminating CAD affected subjects from their unaffected family members selected from the Indian Atherosclerosis Research Study (IARS) as well as test their association with classical cardiovascular risk factors and atherothrombotic biomarkers.

Materials and Methods

Study population

The study population comprise 710 subjects that include 125 CAD affected and 585 unaffected subjects, participating in the IARS. Design of IARS has been previously described [10]. Briefly, CAD patients with premature disease onset (≤ 60 years for men and ≤65 years for women) and unaffected family members were recruited from hospitals in Bangalore and Mumbai, based on predefined inclusion-exclusion criteria and after obtaining written informed consent. Information on demographics, athropometrics, risk factors and medical history was recorded in a standard questionnaire. The study has been designed according to Indian Council of Medical Research (ICMR) guidelines on bioethics [11] and is approved by the local ethics committee.

CIMT data acquisition, measurement and image analysis

Ultrasound images of CIMT were acquired using Sonoline G-50 machine (Siemens Healthcare, Erlangen, Germany) by trained technician, based on the protocol recommended by the American Society of Echocardiography (ASE) [12]. Briefly, B-mode images of right and left common carotid artery, common carotid bifurcation, and proximal part of the internal carotid artery were acquired at end

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diastole. Images were ECG gated and optimized for a good R-wave, digitized to DICOM standards, stored and analyzed off-line. Syngo® Arterial Health Package (Siemens Healthcare, Erlangen, Germany), a semi-automated software, was used to detect intima media boundaries. CIMT was estimated by an experienced ultrasonologist who was blinded to the affected status of study participants.

Assessment of vascular function

Periscope (M/S Genesis Medical Systems, India), an oscillometry based blood pressure monitoring and PC-based acquisition and analysis system was used to measure real time, the following parameters-right brachial PWV (R bra PWV), left brachial PWV (L bra PWV), carotid-femoral PWV (C-F PVW), right brachial ASI (R bra ASI), left brachial ASI (L bra ASI), right ankle ASI (R ank ASI), left ankle ASI (R ank ASI) and ankle brachial index (ABI). All pressure recordings were done for about 10s and the report contained 8-second traces of Lead I and II ECGs and all pressure pulse waveforms.

Measurement of laboratory parameters

Venous blood was collected in evacuated tubes after overnight fast. Serum and plasma aliquots were stored at -80°C and used for measuring Total Cholesterol (TC), Triglycerides (TG) and High Density Lipoprotein-cholesterol (HDL-c) through standard enzymatic method; Low Density Lipoprotein-cholesterol (LDL-c) was calculated using Friedwald's formula [13]. Plasma Fibrinogen and FVII activity (FVII.c) was measured by clotting assay, Interleukin 6 (IL6) by ELISA and high sensitive C reactive protein (hsCRP) using Roche latex kit. Details of manufacturer and the coefficient of variation of biomarkers is given in Supplementary file.

Statistical methods

Routine statistical analysis was carried out on SPSS v 17.0 software (SPSS Inc, Chicago, USA). Age and gender were considered as covariates. Diabetes, hypertension, smoking and dyslipidemia were treated as classical risk factors and body mass index (BMI), waist circumference (WC) and waist hip ratio (WHR) were considered for obesity markers. Presence of metabolic syndrome (MS) [14] and Framingham risk score was assigned using standard international guidelines [15]. Contribution of six risk prediction models that included CIMT, Periscope markers and conventional risk factors (conventional RFs) either alone or in combination to discriminate CAD from unaffected subjects was assessed using Receiver Operating characteristic curve (ROC) analysis, Net Reclassification Index (NRI) and Integrated Discrimination Improvement (IDI) index on 'R' statistical software. Odds ratio (OR) and AUC were expressed along with 95% confidence interval (CI) [16,17]. Detailed statistical methodology is provided in the Supplementary file.

Results

Clinical profile of study population

There were 106 males and 19 females in the CAD affected group and 320 males and 265 females in the unaffected group, respectively. Mean age of affected subjects was significantly higher than the unaffected group, both in males (54.64 ± 1.22 years *vs* 38.88 ± 0.70 years) and females (58.32 ± 2.68 *vs* 43.05 ± 0.72 years). Frequency of diabetes, hypertension, smoking and TC were significantly higher in the CAD group ($p<0.02$) as compared to unaffected individuals. Table 1 shows the clinical profile of the study population.

Carotid intima media thickness and CAD

Data on CIMT was available for 380 subjects of whom 64 were affected (56 males, 8 females) and 316 were unaffected (172 males, 144 females). Mean CIMT and max CIMT of right and left common carotid artery was considered for statistical analysis. Mean ± SE, range and inter tertile range of CIMT is shown in Table S1. Mean IMT (0.68 ± 0.022 mm *vs* 0.52 ± 0.010 mm, $p=8.32 \times 10^{-12}$) and max IMT (0.84 ± 0.026 mm vs 0.66 ± 0.012 mm, $p=3.88 \times 10^{-10}$) were higher in CAD affected as compared to unaffected subjects (Figure 1a). Mean IMT showed 20-fold increased risk of CAD (OR 20.42, 6.13–68.04, $p=8.95 \times 10^{-7}$) when top and bottom tertile were compared and 7-fold greater risk (OR 7.34, 2.11-25.49, p=0.002) when 1st and 2nd tertile were compared before covariate adjustment and OR 6.02, 1.46-24.80, p=0.013 for top versus bottom tertile and OR 4.10, 1.19-

Table 1: Clinical Characteristics of the Study population.

Description	Male (N=426)			Female (N=284)		
	Unaffected (N=320)	CAD Affected (N=106)	p value	Unaffected (N=265)	CAD Affected (N=19)	p value
Age (Mean±SE)	38.88 ± 0.70	54.64 ± 1.22	$1.15*10^{-25}$	43.05±0.72	58.32±2.68	$8.37*10^{-8}$
Diabetes N (%)	34(10.6%)	47(44.3%)	$6.34*10^{-13}$	37(14%)	7(36.8%)	0.016
Hypertension N (%)	38(12.2%)	50(47.2%)	$4.36*10^{-13}$	61(23%)	11(57.9%)	0.002
Smoking N (%)	112(35%)	54(50.9%)	0.003	1(0.4%)	-	
TC (mg/dl)	166.57 ± 2 .08	138.14 ± 3.63	$3.57*10^{-11}$	172.47 ± 2.16	154.00 ± 8.06	0.028
TG (mg/dl)	151.52 ± 4.63	140.15 ± 8.10	0.224	131.52 ± 5.49	160.47 ± 20.51	0.174
HDL –c (mg/dl)	36.55 ± 0.43	35.13 ± 0.75	0.100	41.62 ± 0.58	42.42 ± 2.15	0.720
LDL-c (mg/dl)	100.32 ± 1.72	74.99 ± 2.99	$1.22*10^{-12}$	105.22 ±1.86	79.48 ± 6.90	$3.73*10^{-4}$
Statin	3(3.1%)	55(67.9%)	-	3(3.1%)	11(78.6%)	-
Beta Blocker N (%)	3(3.1%)	41(50.6%)	-	8(8.1%)	12(85.7%)	-
Fibrate N (%)	-	2(2.5%)	-	-	1(7.1%)	-
Calcium Channel Blocker N (%)	1(1%)	16(20%)	-	8(8.1%)	4(28.6%)	-
ACE inhibitor N (%)	3(3.1%)	26(32.1%)	-	8(8.1%)	4(28.6%)	-
Anti platelet N (%)	1(1%)	71(88.8%)	-	2(2.1%)	12(85.7%)	-
Hypoglycaemic agents N (%)	7(7.3%)	27(34.2%)	-	10(10.1%)	3(21.4%)	-
Nitrate N (%)	-	32(40.5%)	-	1(1%)	6(42.9%)	-

HDL: c-High Density Lipoprotein Cholesterol; LDL: c-Low Density Lipoprotein Cholesterol; TC: Total Cholesterol; TG: Triglycerides

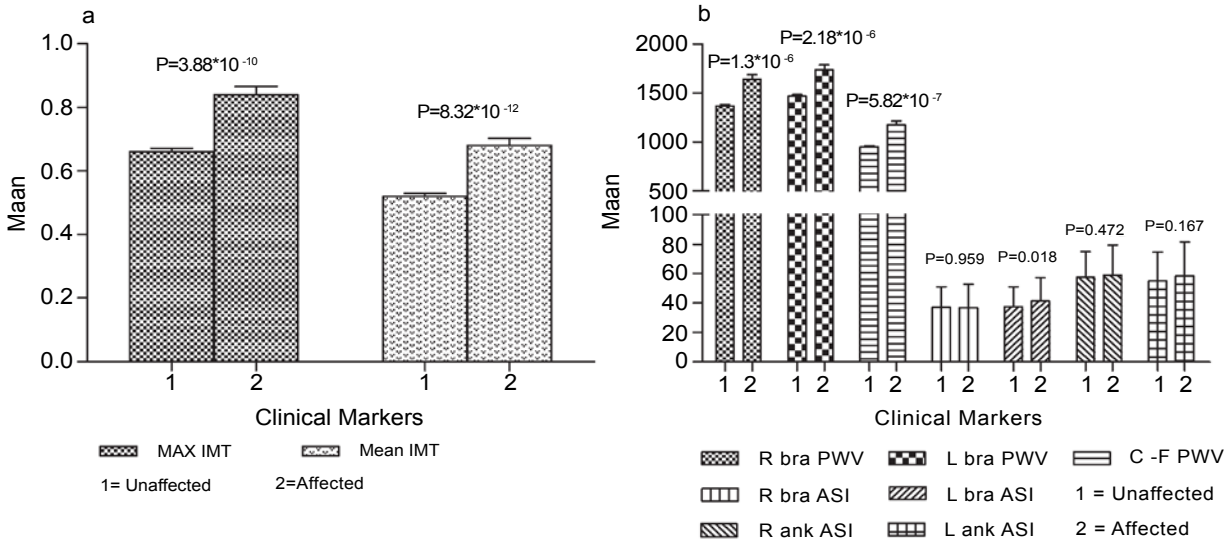

Figure 1: Carotid Intima Media Thickness and CAD.

20.92, p=0.028 for 1st versus 2nd tertile, respectively, after adjusting for covariates. Both mean IMT (0.56 ± 0.01 mm vs 0.52 ± 0.02 mm, p=0.046) and max IMT (0.71 ± 0.01 mm *vs* 0.66 ± 0.12 mm, p=0.047) were higher in males than females.

Periscope markers and CAD

Periscope test was performed in 710 (125 affected, 585 unaffected) subjects. After removing outliers (N=53), data of 657 subjects was included for the final analysis. Mean ± SE, data range and inter tertile range of PWV, ASI and ABI values is shown in Table S2. CAD patients showed higher levels of R bra PWV (1638.23 ± 50.71m/s vs 1367.62 ± 15.02 m/s, $p=1.3 \times 10^{-6}$), L bra PWV (1737.18 ± 51.89 m/s vs 1465.84±18.26 m/s, $p=2.18 \times 10^{-6}$), C-F PWV (1172.99 ± 40.78 m/s vs 949.45 ± 12.49 m/s, $p=5.82 \times 10^{-7}$) and L bra ASI (41.36 ± 15.92 mmHg vs 37.56 ± 13.48 mmHg, p=0.018) than unaffected subjects (Figure 1b). Right bra PWV showed 7-fold increased risk of CAD (OR 7.25, 95% CI 3.87–13.60, $p=6.60 \times 10^{-10}$), when the top and bottom tertile were compared and 3-fold higher risk (OR 3.0, 95% CI 1.54-5.84, p=0.001) between 1st and 2nd tertile. The statistical significance diminished following covariate adjustment. Other variables did not show a significant association. Mean R bra PWV (1457.39±20.82 m/s vs 1351.93±23.63 m/s, p=0.001) and ABI (1.12 ± 0.009 *vs* 1.07 ± 0.01, p<0.001) were higher in males than in females.

Clinical markers with classical cardiovascular risk factors

Mean and max CIMT were significantly higher in presence of diabetes, hypertension, smoking and MS (p<0.005) (Figure S2a) and showed linear association trend with increasing number of risk factors (Figure S2b) and higher FRS groups (Figure S2c). A similar association pattern was seen for PWV (Figures S2d-f) and ASI (Figures S2g-i). Age, BMI, WC and WHR, systolic and diastolic blood pressure showed linear association trend across mean and max CIMT (Table S3), PWV (Table S4) and ASI (Tables S5, S6) tertile, respectively. ABI did not show a significant association with any of the parameters.

Correlation between CIMT and periscope markers

There was strong correlation between mean IMT and max IMT (r-0.92, $p=1.18 \times 10^{-131}$). Significant correlation was also observed among right and left bra PWV and C-F PWV (r=0.70-0.90, $p=1 \times 10^{-101-265}$), between right and left ank- and bra-ASI (r=0.35-0.40, $p=1 \times 10^{-19-28}$) as well as across PWV and ASI (r=0.13-0.31, p=0.001 to 1×10^{-31}). IMT showed correlation mainly with R bra ASI (r=0.142, p=0.01). All clinical markers except ABI showed strong correlation with age (r=0.50, P<0.0001). WC showed higher correlation with CIMT, PWV and ASI (r=0.16-0.27, p<0.0001) than BMI (r=0.10 to 0.14, p<0.01).

Association of clinical indices with atherothrombotic biomarkers

Distribution of various atherothrombotic biomarkers across CIMT, PWV and ASI tertile is shown in Table 2. TC, TG and LDL-c showed significant association with all clinical variables (p<0.03). HDL-c showed inverse association across ASI tertile. FVII.c showed consistent linear association across PWV (p<0.01) and L bra ASI (p=0.021) tertile whereas, lower levels of hsCRP was associated with higher PWV tertile. All associations were adjusted for age and gender.

Association of plaque and effect of statins on clinical markers

Presence of plaque was recorded in 22.2% (14/43) CAD subjects and 4.5% (14/313) unaffected subjects. There was significant association between plaque frequency and CAD ($p=2.18 \times 10^{-5}$). Mean IMT (0.70 mm *vs* 0.53 mm) and max IMT (0.89 mm vs 0.68 mm) were significantly higher in the presence of plaque (p=0.001) for whole data and in unaffected subjects.

CAD patients on statins (N=38) showed reduction in mean CIMT (0.66 mm versus 0.77 mm, p=0.054) and max CIMT (0.82 mm versus 0.95 mm, p=0.055) with borderline significance as compared to those who were not on statins (N=14), respectively. PWV levels were lower in those on statins (1581.45 m/s versus 1792.98 m/s, p=0.113).

Clinical markers and CAD discrimination

AUC for CIMT (0.649, 0.601-0.697) and Periscope (0.722, 0.674-0.770) alone was far lower than for conventional RFs (0.897, 0.870-0.924). Periscope performed relatively better than CIMT, while best AUC value was obtained for model 6 that combined the conventional RFs, CIMT and Periscope markers (0.906, 0.880-0.932) (Figure 2). NRI was not significant whereas IDI was significant for all three combination models. However, the best value was obtained for model 6 (IDI 0.038, 0.019-0.057; p=0.0001). Maximum reclassification of subjects occurred in the intermediate Framingham risk score categories (5-10%, 10-20%) in prediction models 4, 5 and 6.

Discussion

The present study highlights the utility of CIMT, PWV and ASI assessed non-invasively, to significantly discriminate the CAD group, relate to classical cardiovascular risk factors and specific atherothrombotic biomarkers as well as reclassify subjects in the intermediate FRS group (5-20%) under the updated risk prediction model. Our finding of 6-fold higher adjusted risk of CAD using mean IMT as a marker, and 7-fold unadjusted risk using PWV as a marker, implies the promising utility of these vascular indices in predicting future cardiovascular risk. Age has been a strong confounder in this association. CIMT has been reported to be a robust marker for

Table 2: Mean biomarker levels across tertile of clinical variables.

Variable	Biomarkers	1st tertile	2nd tertile	3rd tertile	p value
Max CIMT	TG (mg/dl) (N=109)	121.56 ± 7.01	143.22 ± 7.11	152.62 ± 7.01	0.006
Mean CIMT	TC (mg/dl) (N=109)	156.82 ± 4.23	171.16 ± 3.77	165.68 ± 4.46	0.023
	LDL-c (mg/dl)(N=109)	92.77 ± 3.49	103.42 ± 3.11	97.01 ± 3.52	0.020
L bra PWV	TC (mg/dl) (N=219)	157.16 ± 2.55	167.94 ± 2.56	167.58 ± 2.53	$2.05*10^{-4}$
	TG (mg/dl) (N=219)	121.60 ± 5.04	148.75 ± 5.05	148.13 ± 4.99	0.045
	LDL-c (mg/dl) (N=219)	93.96 ± 2.17	100.96 ± 2.18	98.89 ± 2.15	0.001
	Fibrinogen (g/l)(N=97)	3.75 ± 0.126	3.90 ± 0.095	3.77 ± 0.095	0.002
	FVII C (%NHP)(N=97)	95.27 ± 3.57	103.68 ± 2.69	110.11 ± 2.68	0.010
R bra PWV	TC (mg/dl) (N=218)	157.73 ± 2.93	157.73 ± 2.53	157.73±2.90	$1.67*10^{-4}$
	LDL-c (mg/dl) (N=218)	94.17 ± 2.48	102.09 ± 2.14	97.55 ± 2.46	$2.67*10^{-4}$
	Fibrinogen (g/l) (N=95)	3.92 ± 0.125	3.71 ± 0.098	3.81 ± 0.097	0.012
	FVII.c (%NHP) (N=95)	96.70 ± 3.52	107.72 ± 2.75	106.86 ± 2.74	0.018
	hsCRP ug/dl) (N=73)	4.60 ± 1.02	3.70 ± 0.73	2.67 ± 0.73	0.029
C-F PWV	TC (mg/dl) (N=218)	157.55 ± 2.90	167.92 ± 2.53	167.22 ± 2.91	$2.45*10^{-4}$
	TG (mg/dl) (N=218)	120.55 ± 5.73	148.75 ± 5.00	149.15 ± 5.74	0.038
	LDL-c (mg/dl)(N=218)	94.44 ± 2.46	100.90 ± 2.15	98.46 ± 2.47	0.001
	Fibrinogen (g/l)(N=96)	3.78 ± 0.12	3.88 ± 0.100	3.78 ± 0.098	0.010
	FVII C(N=96)	95.28 ± 3.41	106.43 ± 2.78	108.75 ± 2.74	0.004
	hsCRP(N=73)	4.63 ± 0.99	3.64 ± 0.74	2.72 ± 0.73	0.036
R bra ASI	HDL-c (mg/dl) (N=217)	37.23 ± 0.58	38.22 ± 0.58	39.82 ± 0.58	0.033
	Fibrinogen (g/l) (N=82)	3.95 ± 0.095	3.67 ± 0.104	3.79 ± 0.095	0.011
L bra ASI	FVII.c (%NHP)(N=86)	100.65 ± 2.67	110.13 ± 3.04	104.09 ± 2.62	0.021
R Ank ASI	HDL-c (mg/dl) (N=218)	38.36 ± 0.59	37.45 ± 0.58	39.42 ± 0.59	0.034
L Ank ASI	HDL-c (mg/dl) (N=221)	37.88 ± 0.59	37.43 ± 0.57	39.95 ± 0.59	0.002

C-F PWV: Carotid-Femoral Pulse Wave Velocity; FVII.c: Factor VII Coagulant Activity; HDL: c-High Density Lipoprotein Cholesterol; hs CRP: high sensitive C-Reactive Protein; L Ank ASI: Left Ankle Arterial Stiffness Index; L bra ASI: Left Brachial Arterial Stiffness Index; L bra PWV: Left Brachial Pulse Wave Velocity; LDL: c-Low Density Lipoprotein Cholesterol; Max CIMT: Maximum Carotid Intima Media Thickness, R Ank ASI: Right Ankle Arterial Stiffness Index; R bra ASI: Right Brachial Arterial Stiffness Index; R bra PWV: Right Brachial Pulse Wave Velocity; TC: Total Cholesterol; TG: Triglycerides

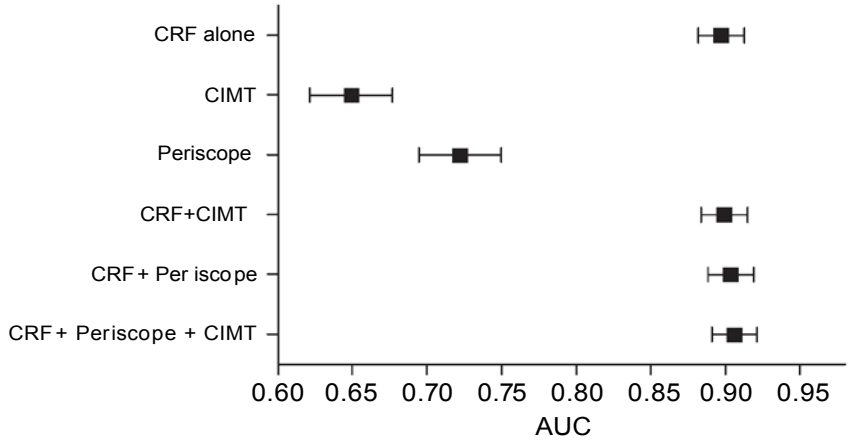

Figure 2: Periscope markers and CAD.

predicting incident CVD or cardiovascular mortality [18,19]. Reports of meta analysis show that relative risk of MI or stroke increases with every 0.1 mm increase in CIMT [1]. Some studies have even rated CIMT as a better index for identifying coronary atherosclerosis than PWV or ABI [20]. CIMT therefore appears to be a strong contender to join the growing list of emerging biomarkers for CVD risk prediction.

Of the three parameters of vascular dysfunction assessed by Periscope, low ABI has shown high specificity for predicting cardiovascular events [2], which was reaffirmed in a systematic review of over 40,000 subjects analysed from 11 different studies [3]. In the present study, however, ABI did not show significant association with CAD probably due to the five fold over representation of unaffected subjects as compared to CAD subjects, which might have masked its true contribution towards discrimination between the affected and unaffected group.

In contrast, PWV showed strong association with CAD and significant correlation with IMT. ABI and PWV are useful indices of arterial stiffening, characteristic of early stages of atherosclerosis, particularly in the peripheral limbs [2]. Indeed, there have been reports on the excellent correlation between enhanced arterial stiffness, PWV and endothelial dysfunction as assessed by reduced brachial artery flow-mediated dilation [4] and high correlation between PWV and CIMT, particularly in CAD patients, which may be indicative of a progressive disease state [8,21].

Established risk factors have shown a close association with CIMT [6,22] and Periscope markers [2,4] which was also seen in the present study [23]. There is ample evidence in literature to suggest that these risk factors can induce early vascular changes. Increased CIMT has been shown to be an independent predictor of deaths in CAD patients with hypertension in a Polish study [6]. Our study as well as other reports has shown a significant increase in systolic BP across both PWV and ASI tertile [24]. Metabolic syndrome has been found to be an independent determinant of CIMT [7] and a significant risk factor for elevated arterial stiffness [25] and PWV [26]. We also observed significant correlation of PWV with abdominal obesity markers, WC and WHR, which was concurrent with other studies [27]. As these classical risk factors generally precede a clinical cardiovascular event, it is but natural that they show a strong relationship with these surrogate markers of endothelia dysfunction. The linear relationship seen between number of risk factors and CIMT, PWV and ASI, has been shown by others as well [22]. We observed that subjects in the higher tertile of IMT and PWV showed elevated levels of TC, TG and LDL-c than in the bottom tertile. Strong association between cholesterol and CIMT has been previously reported in Asian Indians [28]. The interesting association of Fibrinogen and FVII.c with PWV again reflects the influence of pro-coagulant factors in inducing arterial thrombosis, particularly in the narrow peripheral vessels, thereby increasing the risk of developing peripheral arterial disease [29]. In contrast, hsCRP showed inverse association across tertile of PWV. Since data on coagulation markers and hsCRP was available for 100 individuals only, these observations need careful consideration on a larger cohort before drawing definite conclusions

Carotid plaque assessment has been shown to enhance risk prediction in asymptomatic individuals [30]. In our study, asymptomatic subjects with plaque showed higher mean and max IMT. It would be worthwhile to follow-up these individuals prospectively to assess the true relevance of this association. Further, statin treatment has been shown to slow down CIMT progression [31] and improve vascular function [32], compared with placebo. In line with this, we showed that CAD patients on statin therapy showed lower IMT, PWV and ASI though the difference was only marginally significant, which may be attributed to our small cohort size.

We used six different risk prediction models with CRFs, CIMT and Periscope variables used either alone or in combinations thereof. We observed small increments in AUC value between the different model combinations, as reported in other studies [33]. A clinically relevant observation was the substantial reclassification of subjects in the intermediate FRS risk category (5%-20%) across all the combined risk prediction models, which is in line with a recent report from the Rotterdam study [34].

The benefit of CIMT and Periscope assessment are manifold and therefore has strong clinical appeal. They are non-invasive, relatively easy to perform, reproducible and repeated measurements can be taken without adverse biological effects. The key issues of standardized protocols for measurement, analysis and reporting of CIMT has been largely resolved with the publication of a consensus statement by the American Society of Echocardiography in 2008 [12]. The availability of sophisticated software for automated measurement of intima media boundaries has made CIMT reporting less subjective and more reproducible. In the present study, we have followed the ASE guidelines for acquiring the ultrasound images and used dedicated software for image analysis. The Periscope on the other hand, has undergone extensive clinical trials and therefore been validated for repeatability and reproducibility [8,9].

Furthermore, reports of close correlation between endothelial dysfunction in the coronary and peripheral circulation advocate the reliability of CIMT and Periscope markers to serve as surrogate markers of early atherogenesis [8].

We acknowledge certain drawbacks in the study. We have selected a representative subset from the IARS cohort, which is a family-based study wherein, relatedness among family members might have acted as a potential confounder, particularly for CIMT, which shows significant heritability. Also, there is a discrepancy in the number of affected males and females and age between the affected and unaffected group. We have taken care of these differences to some extent by adjusting for age and gender during statistical analysis.

Despite these short-comings, it is important to recognize that these clinical indices have the ability to capture the cumulative effect of multiple risk factors acting at different time points in an individual. Furthermore, combining CIMT with peripheral vascular resistance markers provide a comprehensive assessment of atherosclerotic burden in an individual. Given their strong association with established cardiovascular risk factors, these clinical indices have the potential to add an important dimension to personalized risk assessment in conjunction with emerging molecular markers and more importantly, provide a much awaited handle to monitor disease progression in the asymptomatic 'high risk' younger population. Based on the strength of evidence obtained from the present study as well as other published reports, it may be worthwhile to consider its relevance as a critical tool for identifying the 'at-risk' population.

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Contributorship

The study was designed by JS, SG and VVK. SG was involved in CIMT data analysis, DB helped to enrol the study participants and perform Periscope test, PDV performed CIMT test, VR and VK helped with statistical analysis. JS, VR, VK and VVK contributed to the preparation of manuscript and all approved the final version.

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