# Association of metabolic syndrome with atherothrombotic blood phenotypes in Asian Indian families with premature coronary artery disease

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## Abstract

**Introduction:** Metabolic syndrome (MS) and its co-morbidities including coronary artery disease (CAD) are highly prevalent among Asian Indians and share common risk factors. The aim of this study was to look for an association of atherothrombotic biomarkers with MS among Asian Indian families with premature CAD.

**Material and methods:** MS subjects were identified employing standard and adapted definitions. Lipids were measured on an auto-analyzer while the non-lipid markers were assayed by the ELISA method. Standard and modified MS definitions were applied to 2316 individuals from 531 families.

**Results:** The ATP III criteria identified a significantly larger proportion of people with MS (N=933, P<0.0001) as compared to WHO (N=708) while maximum gain was seen in the modified MS criteria group ($MS_{mod}$, N=1333), which included a lowered cut-off for waist circumference (WC) and body mass index (BMI) (P=0.0056). Atherothrombotic blood phenotypes, namely TC, TG, Ox-LDL, ApoB100, fibrinogen, FVII.c, PAI-1, IL-6, hsCRP, P-selectin, sICAM and leptin, were significantly elevated, while HDL-c and adiponectin were lower in the $MS_{mod}$ group (P<0.01). All the above phenotypes correlated significantly with WC and BMI but not with waist-hip ratio.

**Conlusions:** In conclusion, an association of the atherothrombotic blood phenotypes with MS indicates the propensity of people with MS to develop CAD, thereby emphasizing the need for early and precise diagnosis of MS among Asian Indians.

**Key words:** metabolic syndrome definition, anthropometrics, biomarkers.

## Introduction

Asian Indians have a high incidence of coronary artery disease (CAD). The associated risk factors, namely obesity, insulin resistance, dyslipidaemia and hypertension, have been collectively referred to as metabolic syndrome (MS) [1]. People with MS exhibit a prothrombotic and pro-inflammatory state [2], thus indicating a crossover of common metabolic pathways with CAD. Numerous studies have established the role of inflammatory markers such as C-reactive protein (CRP) in coronary disease progression [3-5]. A strong association has been reported between elevated CRP levels and metabolic syndrome factors, probably mediated

by adipose tissues in obese subjects [6] or by an insulin resistant state [7], which triggers the release of plasma cytokines into the circulation. Inflammation is said to be involved in coronary microcirculation abnormalities commonly seen in patients with MS [8], which might induce the secretion of cell adhesion molecules and growth factors [9]. Increased risk of CVD in individuals with MS may be due to an amalgamation of numerous risk factors including impaired fibrinolysis, primarily mediated by PAI-1 [10]. PAI-1 has been shown to have an in vitro effect on insulin signalling and adipocyte differentiation [11]. Presentation of such a challenged state in MS subjects might eventually set the stage for the early onset of atherosclerosis.

MS diagnosis has been traditionally made using standard guidelines laid down by NCEP-ATP III (National Cholesterol Education Program – Adult Treatment Panel III) [12] and WHO (World Health Organization) [13]. Both definitions have been widely applied to different populations under study. While insulin resistance has been the dominant feature of WHO criteria, waist circumference (WC) rather than body mass index (BMI) has been the differentiating aspect of ATP III. Since Asian Indians have a high propensity to develop MS and its various co-morbidities [14, 15], studies show that MS prevalence is underestimated employing the above standard guidelines. Small build combined with unique fat distribution and predominant abdominal adiposity as compared to Caucasians has instigated the redefinition of basic anthropometric scales for Asian Indians. In fact, WHO has now recognized the need for a population-specific modification of anthropometric measures. The recommended BMI cut-off for defining 'overweight' in Asian Indians is 23 kg/m$^2$ [16], modified waist circumference (WC) measures are ≥90 cm for males and ≥80 cm in females [17] and waist-hip ratio (WHR) is 0.89 for men and 0.81 for women, respectively [18]. These modified cut-offs have been included when studying Asian Indians by several investigators [19-21].

The aim of this study was therefore to understand the association of traditional and novel atherothrombotic biomarkers with metabolic syndrome among Asian Indian families with a strong history of CAD.

## Material and methods

### Study subjects

A total of 531 families with a history of early onset CAD and comprising 2316 individuals were recruited into the **I**ndian **A**therosclerosis **R**esearch **S**tudy (IARS), an ongoing genetic epidemiological study, with an objective to investigate the genetic factors associated with CAD, as also their interaction with traditional risk factors among Asian Indians living in India. Subjects were ascertained through the proband (males ≤60 years, females ≤65 years at onset of CAD) admitted to hospitals and clinics in Bangalore and to the Asian Heart Institute in Mumbai to undergo treatment for CAD and its complications. Other affected and unaffected family members (parents, siblings, spouse and offspring above 18 years) were also enrolled in the study. Demographics, anthropometrics, vital parameters, medical history, medication and pedigree details were recorded for each participant through personal interviews after obtaining written informed consent. Prevalence of type 2 diabetes, hypertension and CVD was ascertained based on self-report of the physician's diagnosis and/or use of prescription medications along with medical records of treatment. None of the probands or family members had concomitant or past major illness. Among the participants, there were 1355 males and 961 females, with a mean of 4.37 individuals/family.

### Metabolic syndrome definitions

MS diagnosis was carried out using the following criteria:

A) *The 2001 NCEP-ATP III guidelines* – require the presence of any three of the following traits in an individual:
   1) abdominal obesity with waist circumference >102 cm in men, >88 cm in women;
   2) serum triglycerides ≥150 mg/dl;
   3) HDL-C ≤40 mg/dl in men, ≤50 mg/dl in women;
   4) blood pressure ≥130/85 mm Hg;
   5) fasting blood glucose ≥110 mg/dl.

B) *WHO criteria:*
   1) insulin resistance (identified by type 2 diabetes mellitus or impaired fasting glucose ≥110 mg/dl) in addition to two or more of the following;
   2) abdominal/central obesity as denoted by waist-hip ratio ≥0.9 in men and ≥0.85 in women or BMI >30 kg/m$^2$;
   3) hypertriglyceridaemia – TG ≥150 mg/dl;
   4) HDL-C <35 mg/dl for men and <39 mg/dl for women;
   5) high blood pressure ≥140/90 mm Hg or documented evidence of anti-hypertensive therapy.

   Microalbuminuria was not assessed in our cohort.

C) *Modified MS criteria* [$MS_{mod}$]*:*

   A modified MS definition was employed by lowering the cut-off values for WC (ATP111) and BMI (WHO) as follows: WC ≥90 cm for men and ≥80 cm for women and BMI >23 kg/m$^2$. Criteria from 2 to 5 were as per the NCEP ATP III guidelines as detailed above.

### Laboratory analysis

Venous blood was collected in evacuated tubes after an overnight fast of 12 to 14 hours (Vacuette®,

Greiner Bio-One GmbH, Vienna, Austria). Serum and plasma aliquots were stored at –80°C until analysis. Fasting venous blood sugar was assayed using a Glucometer (Bayer Diagnostics). Serum triglycerides (Randox Laboratories Ltd., UK), high density lipoprotein-cholesterol (Bayer Diagnostics, Randox Labs and Dade-Behring Limited, UK), total cholesterol and lipoprotein (a) (Randox Laboratories, UK), apolipoprotein A1 and apolipoprotein B100 (Orion diagnostics, Finland) were estimated on a Cobas-Fara II Clinical Chemistry Auto analyzer (F. Hoffman La Roche Ltd, Switzerland). Oxidized-LDL (Mercodia), fibrinogen, FVII.c (Instrumentation Laboratories, Italy), PAI-1 (Diagnostoica Stago), CRP (IBL), hsCRP (Roche Diagnostics, UK), IL-6, sICAM, P-selectin, adiponectin (R&D Systems) and leptin (Bioline) were assayed according to manufacturers' instructions. LDL was calculated using the Friedewald formula. Coagulation parameters were assayed on an ACL 300 (IL systems, Italy) while all other atherogenic biomarkers were assayed by the ELISA method. Lipid and procoagulant factor levels were analyzed for all subjects in the study while non-lipid markers were carried out on a minimum of 500 subjects for each of the assays.

### Statistical methods

Data from the $MS_{mod}$ group were subjected to detailed analysis as the criteria adopted in this group enabled the identification of the highest number of MS subjects. Results are expressed as mean ± standard error of the mean for all continuous variables. Differences in MS prevalence as defined using various criteria were identified employing chi square test. Pearson's partial correlation was carried out to investigate the inter-relationship among MS factors with atherogenic phenotypes after adjustment for gender and age. Quantitative data were assessed for normality of distribution using the P-P Plot and the raw values were log-transformed for normalization of the data. Independent Student's t-test was employed to evaluate the difference in mean levels of various phenotypic markers between those with and without MS. For statistical comparison of continuous variables, ANCOVA was used with adjustment for age, sex, BMI and smoking. A nominal two-sided p-value of $<0.05$ was considered significant. All statistical tests were computed on SPSS v10 software.

The investigation was conducted after obtaining informed consent from participants and under the guidance of the institutional ethics committee.

## Results

Over 2316 CAD affected and unaffected subjects belonging to 531 families were recruited in the Indian Atherosclerosis Research Study (IARS) and categorized as MS and non-MS subjects based on standard and modified definitions. Using standard definitions, a significantly higher number of MS cases were diagnosed using ATP 111 criteria (N=933, 40.3%) when compared to WHO criteria (N=708, 30.6%) ($P<0.0001$). However, the rate of diagnosis was highest in the $MS_{mod}$ group (N=1333, 56.6%). Gender distribution was similar across the $MS_{mod}$ and non-MS groups with 787 (59%) males and 546 (41%) females in the former group and 567 (58.09%) males and 415 (42.5%) females in the latter group, respectively. Peak MS prevalence was in the 50-59 years age group among males and females. The $MS_{mod}$ group was able to identify a larger number of young subjects in the 30-39 year age group across gender as compared to standard definitions.

### MS, DM and CAD

Coronary artery disease was present in 776 (33.5%) individuals in the IARS cohort at the time of recruitment while diabetes mellitus (DM) was prevalent in 1048 (45.3%) subjects. Of the 1333 subjects who presented with MS ($MS_{mod}$), 43.3% (N=577) had CAD, and 68.35% (N=455) had DM. Of those cases with both MS and CAD (N=577), 452 (78.34%) were males and 125 (21.84) were females, respectively. In the non-MS group (N=983), around 20.04% (N=197) had CAD and only 5.5% were diabetic (N=54). Information on diabetic status could not be confirmed in three MS and seven non-MS participants. Over 68.4% (N=455) of subjects in the $MS_{mod}$ group had both DM and CAD as against 30.3% (N=264) with only CAD. The mean age of CAD subjects in our study with or without MS ($MS_{mod}$) and/or diabetes was very similar and was distributed as follows: 57±8 years (presence of CAD, MS and DM), 54.7±9 years (CAD and MS without DM) and 55±11 years (only CAD) respectively. The $MS_{mod}$ criterion was able to diagnose MS in people without CAD at least a decade early (45.8±12.7 years), before CAD onset, as compared to standard definitions.

### MS and atherothrombotic blood phenotypes

The gender-wise distribution of mean plasma levels of atherothrombotic factors under investigation between subjects with MS (N=1333) as defined by $MS_{mod}$ criteria and the non-MS group (N=981) has been provided separately for males and females in Table IA. Total cholesterol, triglycerides, ApoB100 and ox-LDL among the lipids and lipoproteins, fibrinogen, factor V11c among the coagulation factors, markers of inflammation, namely IL-6, CRP, hsCRP, fibrinolytic factor PAI-1, cell adhesion molecules, P-selectin and sICAM and the adipocytokine leptin were significantly higher ($P=0.034$ to $P=0.000$) while HDL-C and adiponectin levels were significantly lower ($P<0.01$), in both male and female subjects with MS when

compared to their non-MS counterparts. However, LDL cholesterol showed a significant difference between MS and non-MS subjects only in females and hsCRP only in males. ApoA1 and Lp(a) did not show a difference in mean levels across MS and non-MS subjects.

Multivariate analysis was employed to test for the effect and interaction between MS, gender and CVD status across all the biomarkers under investigation. There was a significant interaction between MS and CAD affected groups (P<0.01), where TG (0.001), fibrinogen (P=0.054), Lp(a) (P=0.056), P-selectin (P=0.011) and PAI-1 (0.049) were some of the significant contributors to this interaction. TC (P=0.05) was the only factor which made a significant contribution to the interaction between MS and gender.

Among anthropometric measures, WC correlated with WHR (N=1303, r=0.4401, P=0.000) and BMI (N=1303, r=0.7028, P=0.000). However, BMI did not show a correlation with WHR (N=1303, r=0.045, P=0.104). Also, WC and BMI showed a correlation

**Table IA.** Comparison of mean levels of blood atherothrombotic phenotypes between male and female $MS_{mod}$ and non-MS subjects

| Biomarkers | Males | | P value | Females | | P value |
|---|---|---|---|---|---|---|
| | MS yes | MS no | | MS yes | MS no | |
| | mean ± SE (N) | mean ± SE (N) | | mean ± SE (N) | mean ± SE (N) | |
| **Lipids and lipoproteins** | N=687 | N=567 | | N=546 | N=414 | |
| TC [mg/dl] | 167.22±1.49 | 161.6±1.55 | **0.009** | 185.75±1.9 | 169.44±1.97 | **0.000** |
| TG [mg/dl] | 174.28±3.11 | 108.04±2.03 | **0.000** | 153.68±75.58 | 85.23±1.57 | **0.000** |
| HDL [mg/dl] | 36.56±0.29 | 41.86±0.38 | **0.000** | 41.56±0.39 | 48.73±0.58 | **0.000** |
| LDL [mg/dl] | 96.48±1.29 | 98.19±1.37 | 0.364 | 113.09±1.65 | 103.66±1.76 | **0.000** |
| ApoA1 [g/l] | 1.11±0.008 | 1.1±0.009 | 0.562 | 1.21±0.23 | 1.23±0.25 | 0.110 |
| ApoB [g/l] | 1.004±0.01 | 0.88±0.010 | **0.000** | 1.03±0.29 | 0.86±0.011 | **0.000** |
| Lp(a) [mg/l] | 26.19±1.05 (616) | 23.44±1.10 (519) | 0.213 | 28.31±1.40 (499) | 26.43±1.44 (394) | 0.350 |
| OxLDL [mu/l] | 56 915.34 ±1219.55 (209) | 48 985.45 ±1253.55 (146) | **0.000** | 58 611.87 ±1149.98 (187) | 51 025.49 ±2088.08 (79) | **0.001** |
| **Coagulation factors** | **N=783** | **N=566** | | **N=544** | **N=414** | |
| Fibrinogen [g/l] | 3.72±0.03 | 3.37±0.03 | **0.000** | 4.20±0.04 | 3.9±0.04 | **0.000** |
| FV11.c [%NHP] | 105.86±0.85 | 99.69±0.94 | **0.000** | 118.96±0.99 | 106.99±1.16 | **0.000** |
| **Inflammatory markers** | | | | | | |
| IL-6 [pg/ml] | 3.55±0.17 (248) | 2.95±0.21 (158) | **0.028** | 4.20±0.25 (179) | 2.80±0.22 (146) | **0.000** |
| CRP [μg/dl] | 3.21±0.24 (321) | 2.18±0.25 (210) | **0.003** | 5.22±6.10 (224) | 2.89±0.32 (177) | **0.000** |
| HsCRP [μg/dl] | 3.04±0.49 (60) | 1.49±0.27 (47) | **0.007** | 3.5±0.37 (53) | 2.70±0.50 (44) | 0.199 |
| **Cell adhesion molecules** | | | | | | |
| sICAM [ng/ml] | 226.93±3.84 (186) | 210.39±4.73 (136) | **0.007** | 234.56±4.86 (141) | 218.20±5.56 (126) | **0.028** |
| P-selectin [ng/ml] | 49.40±1.15 (203) | 45.60±1.37 (146) | **0.034** | 46.22±1.28 (156) | 41.02±1.22 (130) | **0.004** |
| **Fibrinolytic factor** | | | | | | |
| PAI-1 [ng/ml] | 59.78±2.16 (429) | 40.95±1.65 (294) | **0.000** | 58.79±2.25 (288) | 35.73±1.58 (233) | **0.000** |
| **Adipocytokines** | | | | | | |
| Adiponectin [ng/ml] | 4062.10±176.4 (167) | 5637.76±296.89 (126) | **0.000** | 6238.37±308.99 (130) | 7400.21±353.21 (120) | **0.014** |
| Leptin [ng/ml] | 10.61±0.82 (168) | 8.088±0.79 (126) | **0.000** | 38.78±1.66 (132) | 32.39±1.74 (118) | **0.008** |

*ApoA1 – apolipoprotein A1, ApoB100 – apolipoprotein B100, BMI – body mass index, CRP – C-reactive protein, FV11.c – factor V11 coagulant activity, HDL-c – high density cholesterol, hsCRP – high sensitive C-reactive protein, IL-6 – interleukin 6, lipoprotein (a) – lipoprotein (a), oxLDL – oxidized low density lipoprotein, PAI-1 – plasminogen activator inhibitor 1, sICAM – soluble intercellular adhesion molecule, TG – triglycerides, WC – waist circumference, WHR – waist-hip ratio*

with fibrinogen, FVII.c, IL-6, PAI1, CRP, hsCRP and leptin while WHR did not do so. All the above values were corrected for age and gender (Table IB).

## Discussion

Asian Indians are a high-risk population with respect to DM and CAD and the numbers are consistently on the rise [22]. Standard definitions applied to MS diagnosis do not reflect true population prevalence, thereby delaying disbursement of therapeutic methods to contain this disorder that is reaching epidemic proportions. Various studies have appreciated the ethnic variation in clinical measures and disease outcomes in different populations [15, 23, 24]. The problem partly lies in the cut-off measures used to define obesity in the ATP111 and WHO criteria. Asians Indians have a small build and a unique fat distribution pattern that promotes high insulin resistance and dysmetabolic adipocyte milieu at considerably lower BMI and abdominal adiposity as identified by WC. Both WHO [16] and IDF [17] have recognized the need for a population specific cut-off and recommended revision in BMI and WC measures for accurate MS diagnosis. In the present study, a greater number of MS cases were identified using the revised anthropometric measures ($MS_{mod}$) in comparison to standard definitions. A similar finding was reported by Misra et al. [19]. In fact, several authors have recommended assessment of percent body fat when defining obesity among Asian Indians in order to avoid misclassification in obesity related disorders such as MS [14, 25]. Interestingly, only a small fraction of angiographically proven CAD patients (8.2%) had BMI >27 kg/m$^2$ in an Indian study [26].

The IARS cohort was recruited through probands having premature CAD and a strong family history. MS subjects were identified from this cohort. The peak age of MS prevalence (50-59 years) coincided with the mean age at onset of CAD (51.75±8.62 years). Studies have shown that subjects with MS have a greater risk of cardiovascular complications and death when compared to non-MS subjects [27]. In the present study, modified MS criteria were able to identify a greater number of young people with MS [30-39 years] than standard definitions, which indicates their ability to identify 'high-risk subjects' early, thereby enabling application of prophylactic measures at the appropriate time.

We observed a preponderance of male subjects with MS and CAD (78.34%) as compared to females (21.84%). Male gender has been considered as one of the non-modifiable risk factors for CAD and its co-morbidities. This has been attributed to the protective effect of oestrogen in pre-menopausal women where oestrogen stimulates nitric oxide production, which has multiple beneficial effects on the endothelium including vasodilatation, inhibition of lipid oxidation and monocyte adhesion [28]. A similar effect is lacking among males.

Metabolic syndrome is a pro-coagulant and pro-inflammatory state [2], which is not entirely reflected by the components that define it. There is enhanced secretion of pro-inflammatory cytokines, cell adhesion molecules and growth factors in those with the syndrome. The high levels of several atherothrombotic biomarkers in this study support a pro-inflammatory state in metabolic syndrome. Elevated levels of PAI-1, fibrinogen and FVII.c indicate fibrinolytic dysfunction and pro-thrombotic predisposition among MS subjects. Anand SS et al. have elucidated the relationship of fibrinolytic dysfunction with reference to PAI-1 levels in subjects with MS and CVD [10]. Increased PAI-1 level has been considered as a critical component

**Table IB.** Correlation of anthropometry with non-lipid phenotypes

| Phenotype* | WC | | | BMI | | | WHR | | |
|---|---|---|---|---|---|---|---|---|---|
| | N | r | P value | N | r | P value | N | r | P value |
| Fibrinogen | 1295 | 0.20 | **0.000** | 1295 | 0.12 | **0.000** | 1295 | 0.04 | 0.184 |
| FVII.c | 1295 | 0.09 | **0.001** | 1295 | 0.09 | **0.001** | 1295 | 0.01 | 0.079 |
| IL-6 | 250 | 0.16 | **0.011** | 250 | 0.26 | **0.000** | 250 | 0.01 | 0.882 |
| CRP | 250 | 0.33 | **0.000** | 250 | 0.29 | **0.000** | 250 | 0.16 | 0.095 |
| hsCRP | 598 | 0.26 | **0.000** | 598 | 0.28 | **0.000** | 598 | 0.03 | 0.405 |
| PAI-1 | 242 | 0.27 | **0.000** | 242 | 0.19 | **0.004** | 242 | 0.07 | 0.298 |
| sICAM | 250 | 0.07 | 0.292 | 250 | 0.06 | 0.369 | 250 | 0.05 | 0.465 |
| P-selectin | 250 | 0.16 | **0.010** | 250 | 0.09 | 0.139 | 250 | 0.11 | 0.073 |
| Adiponectin | 250 | –0.11 | 0.082 | 250 | –0.05 | 0.439 | 250 | –0.10 | 0.117 |
| Leptin | 250 | 0.42 | **0.000** | 250 | 0.41 | **0.000** | 250 | –0.03 | 0.693 |

** Correlation computation performed after adjusting for age and gender*

of MS that is capable of acting as a modulator of atherothrombosis and insulin resistance [11].

Anthropometric measures, namely WC, WHR and BMI, are some of the key component criteria for MS diagnosis. The observation that both WC and BMI but not WHR showed a significant correlation ($r=0.1-0.4$, $P\leq0.01$) with many atherothrombotic blood phenotypes (Table 1B) indicates the robustness of using the former two measures rather than BMI as indices of anthropometrics which may thereby serve as good predictors of MS onset and in identifying high-risk CAD subjects. Kurpad et al. also reported a high correlation between WC and BMI and suggest that definition of abdominal obesity using WC is more accurate than using WHR measures [29]. An obese predisposition is generally accompanied by the onset of risk phenotypes common to metabolic syndrome and CAD such as dyslipidaemia, insulin resistance, hypertension, etc. Adipose tissue is now considered as a large endocrine organ, whose secretion, the adipocytokines, have key functions in the metabolic and immunological processes including regulation of vascular endothelial function [30]. PAI-1 has been implicated in the differentiation of adipocytes [31]. High percent body fat has been associated with elevated CRP levels in adolescents and young adults from North India [32]. The role of adiposity in the above context was evident in our study from the differences noted in the levels of adipocytokines wherein high BMI and WC was associated with significantly low adiponectin and high leptin levels in the MS group as compared to non-MS subjects. Reports on the spectrum of actions of leptin are conflicting, showing both beneficial and harmful effects [33-35].

The fact that people with metabolic syndrome and insulin resistance are exposed to elevated risk of both type 2 DM and CAD was apparent in our study, where over 68% of MS subjects manifested with diabetes as well as coronary disease.

All the above findings certainly support a pro-inflammatory, pro-thrombotic and pro-atherogenic potentiation of a person with metabolic syndrome. However, large-scale prospective studies are necessary to pinpoint which of these biomarkers could actually serve as accurate predictors of MS and CVD risk in order to justify their inclusion in the standard risk assessment protocol for the early identification of 'dysmetabolic individuals'.

In conclusion, waist circumference is a preferable marker of abdominal adiposity while BMI appears to be a good predictor of general obesity. The significant association of proatherogenic predisposition with metabolic syndrome explains the substantial propensity of people with MS to develop atherothrombosis and clinical CVD. This emphasizes the need for early recognition and preventive strategies to combat the spectrum of metabolic syndrome and CVD among the high-risk Asian Indian population.

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