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Correlation among soluble markers and severity of disease in non-diabetic subjects with pre-mature coronary artery disease

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Abstract Studies are lacking in literature, which demonstrate the cumulative impact of certain soluble markers in predicting the severity of CAD. Serum hsCRP, MMP-9, TIMP-1 and sRAGE levels were measured in non-diabetic 100 angiographically proven CAD patients (Group I) and 40 non-diabetic subjects with coronary risk factors and without any lesions (Group II). Increased levels of serum hsCRP, MMP-9, TIMP-1 and decreased levels of sRAGE were observed in Group I as compared to Group II. Gensini score, a measure for severity of CAD was found to be positively correlated with serum hsCRP, MMP-9, TIMP-1 and negatively with sRAGE. Multivariate analysis revealed serum MMP-9, hsCRP, sRAGE and family history as predictors of severity of CAD with a cumulative sensitivity and specificity of 92% and 82%, respectively. Cumulative impact of these soluble markers, in addition to the established markers will contribute to improve the predictive value for the assessment of disease severity.

Keywords Pre-mature CAD · sRAGE · MMP-9 · TIMP-1 · Gensini score

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Introduction

Cardiovascular diseases (CVD) are major causes of mortality and disease in the Indian subcontinent, causing more than 25% of deaths. It has been predicted that these diseases will increase rapidly in India and the country will be host to more than half the cases of heart disease in the world within the next 15 years [1]. It usually involves the middle and older age group [2]. Evaluation of major coronary risk factors in Indian patients undergoing angiography has shown that in about one-third of the patients, the disease occurs in absence of any major risk factors [3]. The increasing incidence of CAD in young Indian population (<55 years) is attributed to global industrialization, stressed life, lack of exercise and increasing incidence of smoking and alcohol consumption and other nutritional and lifestyle factors [4].

C-reactive protein (CRP) is an acute-phase reactant, which is a marker for underlying systemic inflammation. There are two different tests for CRP. The standard test measures a much wider range of CRP levels but is less sensitive in the lower ranges. The hs-CRP test can more accurately detect lower concentrations of the protein (as it is more sensitive), which makes it more useful than the CRP test in predicting a healthy person's risk for cardiovascular disease [5]. hsCRP has a useful prognostic utility in patients with myocardial infarction (MI) and unstable angina [6, 7]. Further, several prospective studies have shown that CRP is a predictor of increased risk for MI, stroke or peripheral vascular disease in asymptomatic individuals with no known coronary artery disease [8, 9].

Evidence in literature indicates that serum level of matrix metalloproteinase-9 (MMP-9) levels might be a sensitive inflammatory marker for prediction of cardiovascular mortality in patients with CAD [10, 11], and is

known to contribute to the atherosclerotic lesion progression, plaque vulnerability and de novo atherosclerotic remodelling [12]. MMP activity is controlled by endogenous tissue inhibitors of MMPs (TIMPs). TIMP-1, the best characterized TIMP has been shown to have high prognostic value for the cardiovascular deaths among patients with CVD [13].

The receptor for advanced glycation end products (RAGE) is a multiligand receptor of the immunoglobulin superfamily that engages diverse ligands relevant to the pathogenesis of atherosclerosis. RAGE has a C-truncated secreted isoform, termed soluble RAGE (sRAGE). Differently from cell-surface RAGE, sRAGE blocks cell surface RAGE-ligand binding and subsequent signalling by acting as a decoy [14]. Recently, a couple of studies have reported reduced levels of sRAGE as an important biomarker in hypertension [15], Alzheimer's disease, vascular dementia [16], mild congentive impairment [17] and in non-diabetic subjects with coronary artery disease [18].

Among several classical risk factors for CAD development, oxidative stress and inflammation are now being considered as significant and novel risk factors for CAD and other related manifestations. In literature, several inflammatory mediators have been proven to correlate with the severity or the prognosis of coronary artery disease. From a clinical perspective, the question remains; will the addition of these soluble molecules to the list of already established coronary risk factors improve the predictive value for the risk of CAD? The relationship among circulating levels of hsCRP, MMP-9, TIMP-1, sRAGE and the presence and extent of angiographically defined CAD has seldom been investigated, and such studies are lacking in Indian subjects of CAD. Therefore, in the present study, we for the first time, investigated the correlation among serum sRAGE, MMP-9, TIMP-1, hsCRP and the severity of coronary artery disease in young subjects to evaluate their impact on pre-mature coronary artery disease.

Materials and methods

Study population

Consecutive 140 non-diabetic subjects were enrolled in the present study, who were visiting Cardiology Clinic at Nehru Hospital for coronary angiography owing to coronary artery disease or because of a clinical suspicion of CAD in patients with multiple coronary risk factors. Group I included 100 subjects with angiographically proven CAD. Group II included 40 control subjects, if they had negative treadmill test (TMT) or positive TMT but had no lesion on angiography. Exclusion criteria for both the study groups were as follows: myocardial infarction within 2 months preceding the study, evidence of hemodynamically significant valvular heart diseases, congenital heart disease, surgery or trauma during the month preceding the study, known cardiomyopathy, known malignant diseases, febrile conditions, acute or chronic inflammatory disease, vascular diseases related to the connective tissue disorder, thrombotic diseases on study entry, overt congestive heart failure, renal insufficiency and abnormal liver function. Subjects having a concentration of hsCRP $\geq$10 mg/l, a level considered to be indicative of clinically relevant inflammatory conditions were also excluded from the present study [19].

Pre-mature CAD is defined as CAD occurring before age 65 years in women and age 55 years in men [20]. The mean age for first presentation of acute myocardial infarction in Indians is 53 years, irrespective of sex [21]. Keeping in mind the effects of this epidemic on the productive workforce, we in the present study have recruited subjects of either sex with age $\leq$55 years. Data on demographic factors, smoking habits, alcohol consumption, diet and family history of cardiovascular diseases (hypertension, diabetes, obesity, etc.) in first-degree relative were also recorded. All study participants underwent a standard clinical examination. Body mass index (BMI) was calculated as weight divided by the square of height (kg/m^2). Alcohol intake and cigarette/beedi (a local type of tobacco) smoking was dichotomized into ever versus never, with ever smoking defined as having smoked daily for 1 year or more. Many patients had quit alcohol intake/smoking after onset of CAD; hence, designated as ever rather than current and former. Patients with a systolic blood pressure $\geq$140 mmHg and/or diastolic blood pressure $\geq$90 mmHg (JNC VI definition) or on current use of antihypertensive medication or use of antihypertensive medication for more than 1 year were categorized as hypertensives. The subjects were receiving identical drug therapy including angiotensin converting enzyme (ACE) inhibitors, nitrates, anti-platelets, β-blockers and statins.

The whole study was planned according to the ethical standards detailed in the Declaration of Helsinki [22] and according to the institutional guidelines. Before participation in the study, a written informed consent was obtained from each subject after explaining the protocol. The study was approved by the Institutional Ethics Committee.

Sampling

Venous blood was collected from the overnight-fasted subjects in the morning from anticubital vein into plain sterile tube for serum and in EDTA for plasma just before the angiography and/or after TMT (in subjects who had negative TMT). Serum/plasma was separated and stored at -80°C for further analysis.

Coronary angiography and severity of disease

Coronary arteries are produced as an image in the standard fashion by coronary angiograms. Existing significant CAD was defined as stenosis in the major epicardial coronary arteries that reduced the lumen diameter of ≥50%. The severity of coronary atherosclerosis in patients was assessed by using the Gensini score (GS) [23] which grades narrowing of the lumens of the coronary arteries as 1 for 1–25% narrowing, 2 for 26–50% narrowing, 4 for 51–75% narrowing, 8 for 76–90% narrowing, 16 for 91–99% narrowing and 32 for total occlusion. This score is then multiplied by a factor that takes into account, the importance of the lesion's position in the coronary arterial tree, for example, 5 for the left main coronary artery, 2.5 for the proximal left anterior descending coronary artery (LAD) or proximal left circumflex coronary artery (LCX), 1.5 for the mid-region of the LAD and 1 for the distal LAD or mid-distal region of the LCX. The Gensini score was expressed as the sum of the scores for all coronary arteries.

Measurement of lipid and lipoprotein profile

Total cholesterol (TC) and triglycerides (TG) levels were measured with commercially purchased standard enzymatic kits (Accurex Biomedical Pvt. Ltd.). High-density lipoprotein cholesterol (HDL-C) levels were determined in the supernatant after the plasma was subjected to precipitation with $MgCl_2$ and phosphotungstic acid. Low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein (VLDL-C) values were calculated using Friedwald's formula [24].

Determination of circulating levels of MMP-9, TIMP-1, sRAGE and hsCRP

Circulating levels of MMP-9 (QIA 56, Calbiochem), TIMP-1 (QIA 54, Calbiochem), sRAGE (DRG00, R&D Systems) and hsCRP (RK010A, Hyphen Co., France) were determined in all the study subjects using commercially available enzyme-linked immunoassays (ELISA) as per manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using SPSS 14.0 for Windows (SPSS Inc). The Kolmogorov–Smirnov test of normality was used to verify whether the distribution of variables followed a Gaussian pattern. Data were presented as mean ± SD or percentages. Mean levels of different groups were compared by *t*-test, Mann Whitney U or by analysis of variance (ANOVA) as appropriate. Correlations between variables were analyzed with Pearson's coefficient. Simple (univariate) and multiple (multivariate) logistic regression analysis were performed to determine the association between CAD and all other variables. Receiver operating characteristic (ROC) curves were constructed to determine the optimal values of variables, which provided high sensitivity and specificity.

Results

Anthropometric characteristics

The clinical and laboratory features of the subjects are shown in Table 1. The male:female ratio in Group I subjects was 81:19 as compared to 27:13 in Group II subjects. The two study groups were age and sex-matched and belonged to similar socio-economic status. There were no significant differences between two study groups regarding age, BMI, smoking and alcohol intake, TC and HDL-C ($P > 0.05$), whereas TG levels were found to be significantly higher in group I subjects ($P < 0.05$). We observed no significant difference in the mean systolic blood pressure (SBP) recordings ($P > 0.05$) in the two groups, whereas diastolic blood pressure (DBP) recordings were found to be significantly higher in the patient group subjects ($P < 0.05$). Group I subjects with CAD had significantly higher levels of MMP-9 ($P < 0.001$), hsCRP ($P < 0.001$) and TIMP-1 ($p < 0.05$), when compared to the serum levels in Group II subjects. However, sRAGE levels were found to be significantly decreased in CAD subjects, when compared to their control counterparts ($P < 0.001$; Table 1).

Comparison of MMP-9, TIMP-1, sRAGE and hsCRP in sub-groups of CAD subjects

To address the clinical need for improved assessment of risk among CAD subjects, we performed a sub-group analysis.

Comparison of MMP-9, TIMP-1, sRAGE and hsCRP in CAD subjects sub-grouped on the basis of smoking

When these patients were sub-grouped on the basis of smoking, except sRAGE, all the other variables (MMP-9, TIMP-1 and hsCRP) were found to be insignificantly different between CAD patients who were smokers versus non-smokers. However, sRAGE levels were found to be significantly lower in CAD subjects who were smokers ($n = 47$; 784.32 ± 462.42 pg/ml) as compared to the non-smokers ($n = 53$; 988.23 ± 532.5 pg/ml) ($P < 0.05$).

Table 1 Baseline characteristics of all the study subjects

Variables	Group I ($n = 100$)	Group II ($n = 40$)	P
Age (years)			
Range	24–55	24–55	NS
Mean ± SD	44.4 ± 7.9	41.6 ± 7.5	NS
Sex ratio (M:F)	81:19	27:13	NS
BMI (kg/m^2)	22.68 ± 1.95	22.55 ± 2.95	NS
Blood pressure			
SBP (mmHg)	125.47 ± 10.93	121.95 ± 9.21	NS
DBP (mmHg)	81.65 ± 9.19	77.50 ± 7.42	0.012*
Lipid and lipoprotein profile			
TC (mg/dl)	190.01 ± 35.99	179.45 ± 29.65	NS
TG (mg/dl)	153.71 ± 56.60	132.71 ± 39.29	0.034*
HDL (mg/dl)	42.87 ± 9.14	44.92 ± 8.4	NS
LDL-C (mg/dl)	116.34 ± 32.57	107.98 ± 28.77	NS
Soluble markers			
hsCRP (μg/ml)	5.76 ± 3.12	1.68 ± 1.9	<0.0001***
MMP-9 (ng/ml)	44.31 ± 19.93	15.74 ± 7.69	<0.0001***[b]
TIMP-1 (pg/ml)	279.29 ± 153.93	226.53 ± 84.58	0.043*
sRAGE (pg/ml)	892.39 ± 508.68	1611.90 ± 677.31	<0.0001***[b]
Risk factors			
Hypertension (%)	31	0	–
Smoking (%)	47	38	NS[a]
Alcohol intake (%)	28	35	NS[a]
Medications			
Aspirin	63	0	–
Nitrates	32	0	–
Calcium channel blockers	44	0	–
ACE Inhibitors	46	0	–
Angiotensin receptor blockers	14	0	–
Beta blockers	39	0	–
Statins	71	0	–

Data expressed as mean ± SD and percentage

n number of subjects, *NS* non-significant

[a] Chi-square test

[b] Mann Whitney U test

* $P < 0.05$; *** $P < 0.001$

Comparison of MMP-9, TIMP-1, sRAGE and hsCRP in CAD subjects sub-grouped on the basis of alcohol intake

Similar to the data observed for the smokers, sRAGE was found to be the only variable among all other variables, which was significantly different ($P < 0.05$) between alcoholics ($n = 28$; 710.0 ± 431.23 pg/ml) and non-alcoholics ($n = 72$; 963.32 ± 521.41 pg/ml).

Comparison of MMP-9, TIMP-1, sRAGE and hsCRP in CAD subjects sub-grouped on the basis of presence or absence of hypertension

Further, we categorized CAD subjects on the basis of absence ($n = 69$) and presence ($n = 31$) of hypertension. We observed that TIMP-1 levels were significantly higher in hypertensive CAD subjects (328.52 ± 179.98 pg/ml) as compared to those CAD subjects who were non-hypertensive (257.16 ± 136.4 pg/ml) ($P < 0.05$). sRAGE levels were also found to be significantly different between both these sub-groups ($P < 0.05$) (CAD^+HTN^-:CAD^+HTN^+::978.19 ± 561.32:701.42 ± 291.75 pg/ml). However, levels of hsCRP and MMP-9 were comparable in these two sub-groups and the differences were not significant ($P > 0.05$).

However, we observed no significant difference in any of the parameters considered in this study, when sub-grouped on basis of presence or absence of family history and intake of drugs.

Statistical analysis

Correlation analysis among MMP-9, TIMP-1, hsCRP, sRAGE and severity of CAD

We determined circulating levels of MMP-9, TIMP-1, hsCRP and sRAGE in all the CAD patients using

Table 2 Associations between soluble markers and severity of coronary artery disease as determined by Pearson's correlation analysis

Variable	GS	MMP-9	TIMP-1	sRAGE	hsCRP
GS	1	0.533**	0.513**	−0.342**	0.547**
MMP-9	0.533**	1	0.376**	−0.419	0.341**
TIMP-1	0.513**	0.376**	1	−0.250*	0.223*
sRAGE	−0.342**	−0.419**	−0.250*	1	−0.326**
hsCRP	0.547**	0.341**	0.223*	−0.326**	1

GS Gensini score

* $P < 0.05$; ** $P < 0.01$

immunoassay and further correlated these levels with the severity of CAD. We found that Gensini score, a measure of severity of CAD, was positively and significantly associated with MMP-9 ($r = 0.533$; $P < 0.01$), TIMP-1 ($r = 0.513$; $P < 0.01$) and hsCRP ($r = 0.547$; $P < 0.01$); whereas sRAGE was found to be negatively correlated with the severity of CAD ($r = -0.342$; $P < 0.01$; Table 2).

Logistic regression

The results of logistic regression analysis are presented in Tables 3–4 to evaluate significance of each variable in Table 1. There were significant differences between the two groups in terms of MMP-9, sRAGE, hsCRP levels and family history. Multiple logistic regression analysis after controlling for MMP-9, sRAGE, hsCRP and family history

Table 3 Univariate logistic regression analysis

	SE	*P*	Odds ratio	95.0% CI for odds ratio	
				Lower	Upper
Smoking	1.180	0.049	10.221	1.011	103.352
FH	1.176	0.006	25.131	2.509	251.699
MMP-9	0.030	0.003	1.093	1.032	1.158
sRAGE	0.001	0.016	0.998	0.997	1.000
hsCRP	0.169	0.010	1.549	1.112	2.158

FH presence of family history for cardiovascular diseases, *SE* standard error

Table 4 Multivariate logistic regression analysis

	SE	*P*	Odds ratio	95.0% CI for Odds ratio	
				Lower	Upper
FH	0.831	0.048	5.169	1.014	26.360
MMP-9	0.026	0.002	1.087	1.032	1.145
sRAGE	0.001	0.022	0.999	0.998	1.000
hsCRP	0.151	0.003	1.557	1.158	2.094

FH presence of family history for cardiovascular diseases, *SE* standard error

demonstrated that both sRAGE and MMP-9 were independently correlated with CAD ($P < 0.001$). The other independent predictors of CAD were the presence of family history of CVD and hsCRP. However, no significant association was observed with other parameters e.g. gender, alcohol intake, smoking, lipids and lipoproteins.

Further, multivariate logistic regression revealed that presence of family history of CVD and levels of hsCRP, MMP-9 and sRAGE cumulatively are the best predictors of CAD with 92% sensitivity and 82% specificity.

As MMP-9, hsCRP and sRAGE achieved significance, ROC curves were constructed to determine the optimal values, which provide high sensitivity and specificity. The area under the curve (AUC) was determined, which was significant for MMP-9 (AUC = 90.4%, $P < 0.001$), hsCRP (AUC = 87.4%, $P < 0.001$) and sRAGE (AUC = 81.4%, $P < 0.001$). The sensitivity and specificity were calculated for each possible threshold value of estimated probability for the respective group. Cut-off value for MMP-9 levels, which achieved an optimal sensitivity of 88% and specificity of 77.5% was 20.1 ng/ml, whereas for hsCRP levels, 87% sensitivity and 70% specificity was achieved at a cut-off of 2.06 μg/ml. Similarly, sRAGE levels achieved an optimal sensitivity of 72.5% and specificity of 77% at a cut-off value of 1,088.5 pg/ml for prediction of CAD in these subjects with pre-mature coronary artery disease (Fig. 1).

The patients with CAD and control subjects were further categorized in quartiles based on the variables which were found to be significant in multivariate analysis. We observed that serum sRAGE was the only variable and not the serum levels of MMP-9 and hsCRP (though, both have high levels of significance as compared to the sRAGE), which demonstrated a significant difference in differentiating CAD patients from control subjects across the quartiles. The inter-quartile cut-off points of levels of sRAGE were categorized in four categories i.e. 1–4. The inter-quartile cut off points of serum sRAGE levels were 607 pg/ml, 891 pg/ml, 1518 pg/ml; category 1, <607 pg/ml; 607 pg/ml ≤ category 2 <891 pg/ml; 891 pg/ml ≤category 3 <1518 pg/ml; category 4 ≥ 1518 pg/ml.

The numbers of subjects (in terms of percentage), according to the quartiles of sRAGE concentration are shown in Fig. 2. In the first quartile (sRAGE concentration <607 pg/ml), the number of CAD patients were 13.6-folds higher that of the control subjects ($P < 0.001$). However, the number of CAD patients in the fourth quartile (sRAGE concentration ≥1518 pg/ml) were 3.3-times lower than that of the control subjects ($P < 0.001$).

To evaluate the risk associated with decreasing levels of sRAGE, we calculated odds ratio (ORs) for each quartile (based on the entire study cohort) relative to the fourth quartile. As shown in the Table 5, the ORs for CAD in the first and second quartile of sRAGE levels were

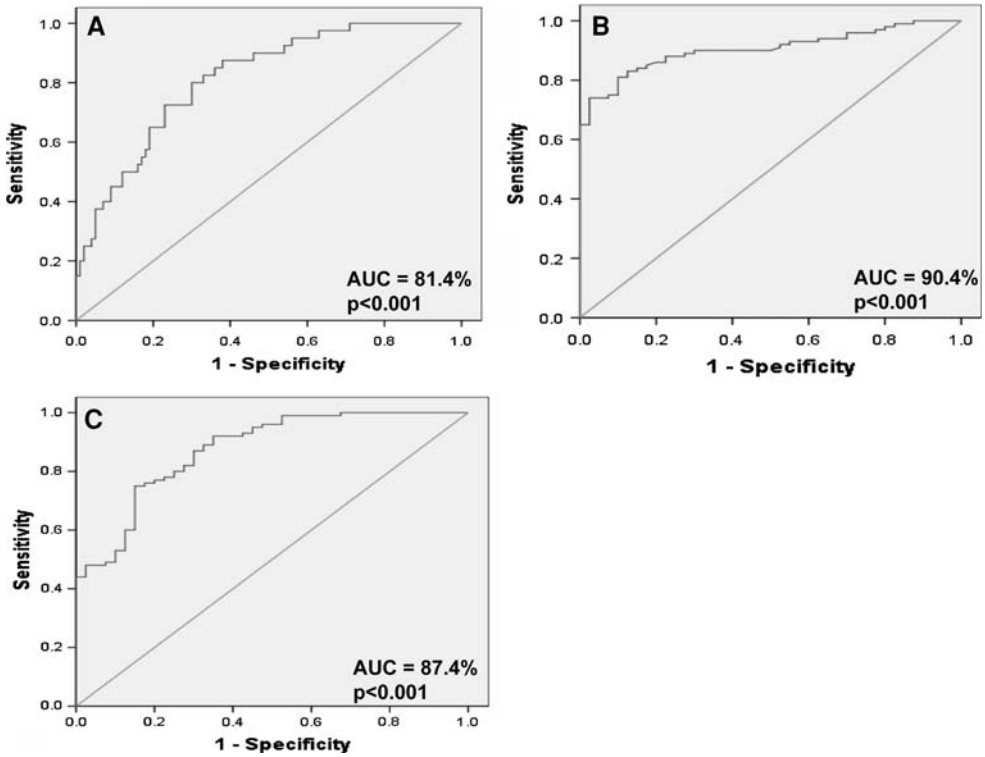

Fig. 1 Receiver operating characteristic (ROC) curves for sRAGE (**a**), MMP-9 (**b**) and hsCRP (**c**) for prediction of severity of coronary artery disease

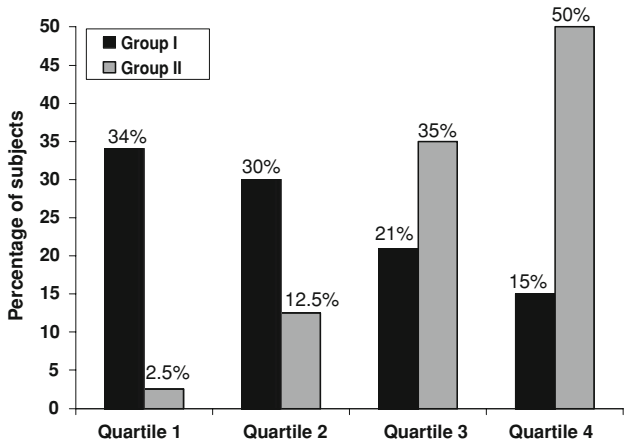

Fig. 2 Percentage of subjects across quartiles of sRAGE levels. Percentage of subjects across increasing quartiles of sRAGE levels in Group I and Group II subjects. Quartile 1 = <607 pg/ml; Quartile 2 = ≥607 to <891 pg/ml; Quartile 3 = ≥891 to <1,518 pg/ml; Quartile 4 ≥1,518 pg/ml

Table 5 Odds ratio for the prevalence of CAD in each quartile of sRAGE concentration relative to the fourth quartile

	Odds ratio	95% CI		*P*
		Lower	Upper	
Category 1 vs. 4	45.333	5.561	369.249	<0.001***
Category 2 vs. 4	8.000	2.509	25.507	<0.001***
Category 3 vs. 4	2.000	0.772	5.180	0.153

CI confidence intervals

Category 1 = sRAGE levels <607 pg/ml; Category 2 = ≥607 to <891 pg/ml; Category 3 = ≥891 to <1,518 pg/ml; Category 4 ≥1,518 pg/ml

*** $P < 0.001$

significantly higher as compared with the fourth quartile ($P < 0.001$). However, no significant difference was observed between the third and fourth category ($P > 0.05$).

Discussion

A very important cardinal feature of CAD in Indians is its marked pre-maturity and severity. A high rate of CAD in Indians is attributed to nature (genetic predisposition) and nurture (environment). It has been projected that by the year 2010, 60% of the world's population with heart disease will be in India [1]. According to the epidemiological data reported by Enas et al. [25] and Indrayan [26], cases of CVD may increase from about 2.9 crore in 2000 to as many as 6.4 crore in 2015, and the number of deaths from CVD will also be more than double. Data also suggest that although the prevalence rates of CVD in rural populations will remain lower than that of urban populations, they will continue to increase, reaching around 13.5% of the rural population in the age group of 60–69 years by 2015. Also, the prevalence rates among younger adults (age group of 40 years and above) are also likely to increase; and the

prevalence rates among women will keep pace with those of men across all age group. As far as the scientific literature is concerned, there is considerable lack of evidence for correlations which exist especially in young subjects who are suffering from pre-mature coronary artery disease.

In the present study, the sex distribution in CAD subjects was 81% males and 19% females, whereas it was 77% males and 23% females in the controls (Table 1). The study was predominantly male oriented as CAD affects young males more severely and commonly than females, which can be attributed to the protective effect of estrogen in females [27].

Tobacco smoking is one of the most powerful modifiable risk factor for the development of CAD. Univariate analysis in CAD subjects revealed that subjects who were smokers had 10 times more risk for development of CAD as compared to those patients who were non-smokers. Smoking cessation may thus prove to be one of the most cost-effective approaches in both primary and secondary preventions. Further, a positive family history, as a non-modifiable risk factor contributed significantly in the pathogenesis of CAD with a relative risk of 25.13 (95% CI 2.5–251.6; $P < 0.006$; Table 3), independently of the other known risk factors for pre-mature CAD in these subjects, thus, suggesting important additive effects of genetic and modifiable risk factors.

We observed significantly higher levels of hsCRP in patients with pre-mature CAD as compared to the risk control group and further, a significant and positive correlation between levels of hsCRP and the severity of CAD in Group I subjects. Our data supports the findings of Haidari et al. [28] and Tataru et al. [29] who independently reported a positive correlation of increased hsCRP levels with the severity of the disease in subjects with stable coronary artery disease and in subjects with acute coronary syndrome (ACS). These findings further highlight the importance of hsCRP as a marker of disease activity. On the contrary, a couple of other studies by Rifai et al. [30] and Abdelmouttaleb et al. [31] did not demonstrate any significant correlation of hsCRP with the severity of CAD, though they also observed increased hsCRP levels in similar set of subjects. Further, we did not observe any significant correlation of hsCRP levels with any of the other classical risk factors like age, BMI, lipids and lipoproteins in Group I subjects. Our reports are contrary to the findings of Mendall et al. [32] and Tracy et al. [33] who reported a positive association of hsCRP with age and BMI in 1,395 and 400 subjects with coronary heart disease, respectively. The reason for these discrepancies may be attributed to the differences in population characteristics and the sample size in these studies. Thus, our results reconfirm the existing relationship between high CRP levels and presence of CAD as documented by coronary angiography. Our findings also indicate the additive value of hsCRP measurement in the coronary risk assessment among a population from a developing country with high prevalence of CAD. Different studies of both cross-sectional and case-control designs have also shown a strong association between CRP levels and the risk of CAD [28, 34, 35].

MMPs have been implicated in vascular remodelling process and have been shown to play significant role in plaque formation and plaque rupture, thereby contributing to the pathogenesis and progression of atherosclerosis [12]. Similar to the previous reports by Noji et al. [36] and Kalela et al. [37], we also observed elevated levels of MMP-9 in Group I subjects and our data demonstrated a significant correlation of MMP-9 with the severity of coronary artery disease. In contrast, Tayebjee et al. [38] reported no significant correlation between MMP-9 and disease severity in angiographically proven peripheral arterial disease with intermittent claudication and critical ischemia. Similarly to the observations with MMP-9 and hsCRP levels, TIMP-1 levels also increased in CAD subjects and showed a positive and significant correlation with the Gensini score, MMP-9 and hsCRP levels. TIMP-1 is a potent inhibitor of MMP-9, and increase in TIMP-1 levels during acute phase of CVD may indicate the induced production of MMP-9 in the affected area. Increased synthesis of TIMP-1 may occur to counterbalance the MMP activity (i.e. a negative feedback pathway) and has been proposed as a possible mechanism of action of TIMP-1 in experimental studies [39].

sRAGE has been documented as anti-atherogenic molecule, and low levels of sRAGE were found to be significantly associated as a risk factor for CAD in a non-diabetic Italian male population [18]. In the present study also, a significant and independent association of decreased levels of sRAGE with presence of coronary artery disease was observed, which further corroborates above findings. A negative correlation of sRAGE with MMP-9, hsCRP, TIMP-1 and Gensini score provides further justification to the anti-atherogenic nature of this molecule. Statistically, sub-group analysis based on the gender difference did not show any significant difference in sRAGE levels between male and female subjects. We also observed that CAD subjects who were hypertensive had low levels of sRAGE as compared to those CAD subjects who were normotensive. These observations are consistent with earlier observations of Geroldi et al. [15], where they demonstrated that levels of sRAGE were decreased in patients with hypertension and raised the possibility that sRAGE may play a role in arterial stiffness and its related complications. Our data demonstrated absence of any significant correlation of sRAGE with BMI. However, Koyama et al. [40] reported a significant inverse correlation of sRAGE and BMI.

Though, exact reason for this is not known but the variation in ethnic population and in the age difference of the subjects in these two studies may account for these variations, as our subjects were of relatively young age with pre-mature CAD and belonged to Indian population. The multivariate ORs for CAD revealed that subjects with sRAGE levels below 607 pg/ml had a 13.6-fold increase in CAD prevalence, independent of the other factors.

As the present study is correlational, it does not allow us to draw a proven mechanistic conclusion. However, based on our findings, we strongly consider that circulating level of MMP-9 is the best predictor for CAD in Indian subjects with high sensitivity and specificity. However, when the CAD subjects were categorised on the basis of either presence or absence of risk factors and across the quartile, only sRAGE levels were able to discriminate between the groups, though sensitivity and specificity of sRAGE was lower than MMP-9. Further, our data for multivariate logistic regression analysis demonstrates that the presence of family history for CVD, levels of hsCRP, MMP-9 and sRAGE cumulatively are the best predictors of CAD with 92% sensitivity and 82% specificity. However, some limitations of this study should also be considered. Firstly, we have not taken samples of these subjects at the time of their follow-up; therefore, we are not able to comment on the effect of drugs on the variables under consideration. Second limitation of the study is that the patients enrolled in this study were only from the north Indian population, thus caution should be exercised for extrapolating the data to other ethnic groups. Lastly, the ELISA detection system used in the present study could not discriminate between specific soluble RAGE variants (sRAGE and esRAGE).

The measurement of these molecules extends the prognostic information gained from traditional classical biochemical markers i.e. lipids and hsCRP. These findings also strengthen the evidence that an inflammatory process is critical in the pathogenesis of coronary artery disease and suggest that the intensity of the inflammatory response can influence the clinical outcome of the disease. These observations have strong implications for clinical practice as inflammatory markers may facilitate risk stratification of these patients during the acute-phase of disease presentation. In nutshell, current data clearly demonstrates that cumulative output of these soluble markers is of greater importance in assessment of the severity of CAD than the product of the individual risk factor alone.

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