Association Of Serum Advanced Glycation End-Products And High Sensitivity C-Reactive Protein Levels With Quantitative Coronary Angiography Derived Severity And Extent Indices In Coronary Artery Disease Patients With Multiple Cardiac Risk Factors

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ABSTRACT

Objective: *Advanced glycation end-products (AGEs) impart contribution in promoting atherosclerosis by means of structural protein changes in the vascular wall and by activation of pro-inflammatory pathways. Thus, study was maimed at evaluating the possible association of serum AGEs and inflammatory marker high sensitivity C-reactive protein (hsCRP) levels (measured on the day of angiography) with quantitative coronary angiography (QCA) derived severity indices, and this may provide additional screening tool in predicting Cardiovascular disease (CVD) risk.*

Methods: *114 patients (age ≥18years) undergoing coronary angiography during January to July 2011, for diagnosis and/or interventional treatment for CAD at Sadbhavna Medical and Heart Institute, Patiala (India) were investigated. Patients were divided into two groups based on the presence of no. of risk factors (either 3 or >3). 40 healthy control samples were also taken. Overnight fasting serum samples for estimation of AGEs and hsCRP levels were taken, analyzed and correlated with angiographic markers of CAD severity (i.e. severity index, extent of severity and number of stenosed vessels)*

Results: *Serum levels of AGEs and hsCRP were significantly higher in CAD patients as compared to healthy control (all p<0.001). AGEs and hsCRP correlated well with angiographic CAD severity, extent and number of stenosed coronary arteries (all, p<0.001). At multivariable regression analysis, age, hypertension, AGEs and hsCRP were all independent determinants of CAD. Moreover, male gender, age, hypertension, AGEs and hsCRP remained independently associated with three-vessel disease (TVD).*

Conclusion: *AGEs and hsCRP levels act as independent predictors of extent & severity in CAD.*

Key words: coronary artery disease, multiple cardiovascular risk factors, advanced glycation end- products, high sensitivity C-reactive protein, cardiovascular disease risk.

INTRODUCTION

Coronary Artery Disease (CAD) is the single most important disease in the world in the terms of mortality, morbidity, disability and economic loss (Gazino 2005). The presence and the number of CAD risk factors predict the future cardiovascular events in individuals with such factors (Hatmi *et al.,* 2007). Advanced glycation end products (AGEs), are a heterogeneous group of compounds non- enzymatically formed in the body during oxidative stress, act as pro-inflammatory mediator (Huebschmann et al., 2006). Increased serum level of AGEs may be associated with atherosclerosis (Kanauchi *et al.,* 2001). AGEs contribute their effects partially through "receptor for AGEs" (RAGE); by binding with its ligands triggers RAGE-dependent cellular activation, leading to endothelial activation and pro-atherogenic vascular changes (Lu *et al.,* 2006).

Inflammation has central role in atherogenesis and inflammatory marker (high-sensitivity C-reactive protein (hsCRP) is a known downstream effector of advanced glycation end-products (Cohen *et al.,* 2006; Ross 1999). We hypothesized a direct relationship of AGEs and hsCRP with angiographic indices of CAD and a direct relationship of hsCRP with AGEs and CAD indices. In addition, since the formation of advanced glycation end products can be promoted by inflammation (Lu *et al.,* 2006; Koyama *et al.,* 2005), patients with CAD or atherosclerotic risk factors were included in the present study for comparison with non-CAD patients. Thus, study was aimed at evaluating the possible association of serum levels of AGEs and hsCRP with the

severity and extent of CAD using quantitative coronary angiography (QCA) in the patients of CAD with multiple cardiovascular risk factors.

METHODS

Study population

This study included patients undergoing coronary angiography between December 2010 and June 2011 for the diagnosis and interventional treatment of CAD in Sadbhavana Medical and Heart Institute, Patiala, Punjab, India. Briefly, from a total of 207 consecutive patients, patients with two or less than two risk factors (*n*=74), congenital heart disease (*n*=6), or cardiomyopathy (*n* =3) were excluded. Exclusion also included patients refusing to give informed consent to take blood sample (*n*=12). Patients were subdivided into two groups based on the presence or absence of number of cardiovascular risk factors and significant coronary artery stenosis (luminal diameter narrowing ≥70%, a severity level previously correlated with reduced coronary flow reserve and is commonly used (Scanlon *et al.*, 1999). Accordingly, group I consisted of 60 patients (56 men, 4 women, mean age, 51.94±0.98 years) of significant CAD with three cardiovascular risk factors. Group II included 65 patients (60 men, 6 women, mean age, 51.03±0.68 years) of significant CAD with more than three risk factors. Further groups were further classified with respect to the number of diseased coronary arteries (1-, 2-, or 3-vessel disease), according to the Consensus definitions of the American Heart Association and the American College of Cardiology. All the patients included were non-smokers. Healthy age and sex matched control subjects (n=45) were also taken. The protocol was approved by the local institutional Ethics Committee of Punjabi University, Patiala (India) and performed in accordance with the declaration of Helsinki and the code of Good Clinical Practice after obtaining a written informed consent from all participants.

Coronary angiography and quantitative analysis

Significant CAD was diagnosed visually if luminal diameter narrowing was estimated as ≥70% in a major epicardial coronary artery. Left main coronary artery narrowing ≥50% was considered as 2-vessel disease. QCA was performed using the Cardiovascular Measurement System by an interventional, highly experienced cardiologists appointed to the study protocol. The severity index was defined as the average of the most severe stenosis in the left main, left anterior descending, left circumflex, and right coronary arteries. The extent index was calculated

as the longitudinal percentage of coronary segments involved with a stenosis (Lu *et al.*, 2006; Pajunen *et al.*, 1997).

Extent Index = (100×∑ [stenosis lengths]/∑ [segment lengths])

Biochemical assessments

Blood was collected after an overnight fasting in all patients, after 24 h of symptom onset for those presenting with an acute coronary syndrome and on the same day of coronary angiography. Serum AGEs levels were determined fluorimetrically by using ELICO SL-174 spectrofluorimeter (Munch *et al.*, 1997). Serum hsCRP was determined by an ultra-sensitive, quantitative turbidimetric test using Accucare hsCRP Terbilatex kit (Lab-Care Diagnostics (INDIA) Pvt. Ltd.), with a linearity range of 2-150 mg/L and an inter-assay CV<6%. Blood HbA1c concentration was measured by using Cation-Exchange Method using Glycosylated Hemoglobin (A1-Fast Fraction) kit (Medsource Ozone Biochemicals Pvt. Ltd., INDIA). Determination of blood glucose by GOD-PAP method using Glucose (S.L) kit (Agappe Diagnostics Ltd., INDIA), total cholesterol and HDL by enzymatic method using Liquizone Cholesterol & HDL Cholesterol-MR kit, triglycerides by enzymatic method using Liquizone Triglycerides-MR kit (Medsource Ozone Biochemicals Pvt. Ltd., INDIA), and LDL by Enzyme selective protection method using LDL-C Direct kit (Agappe Diagnostics Ltd., INDIA) were done, respectively. All the determinations were carried out by using Erba Manheim Chem-5 plus v2 (Model: EC-5 plus v2) auto analyzer.

Statistical analysis

All data were expressed as mean ± SEM. Comparisons of AGEs, hsCRP and other factor values among groups were done by one-way ANOVA, with post-hoc analysis in two-group comparisons performed by the Fisher PLSD test. Spearman's correlation coefficient was used to assess the correlation between biochemical measurements and the number of diseased vessel. Pearson's correlation analysis was used to find the association between continuous variables (e.g., CAD severity and extent indices) and biochemical measurements. Stepwise multivariable linear regression analysis was performed to determine independent risk factors for developing CAD and stepwise multivariable logistic regression analysis was performed to determine independent risk factors for 3-vessel disease in CAD. We used two models in multivariable regression analysis. In model 1, traditional epidemiological risk factors (Table 1) were used; in

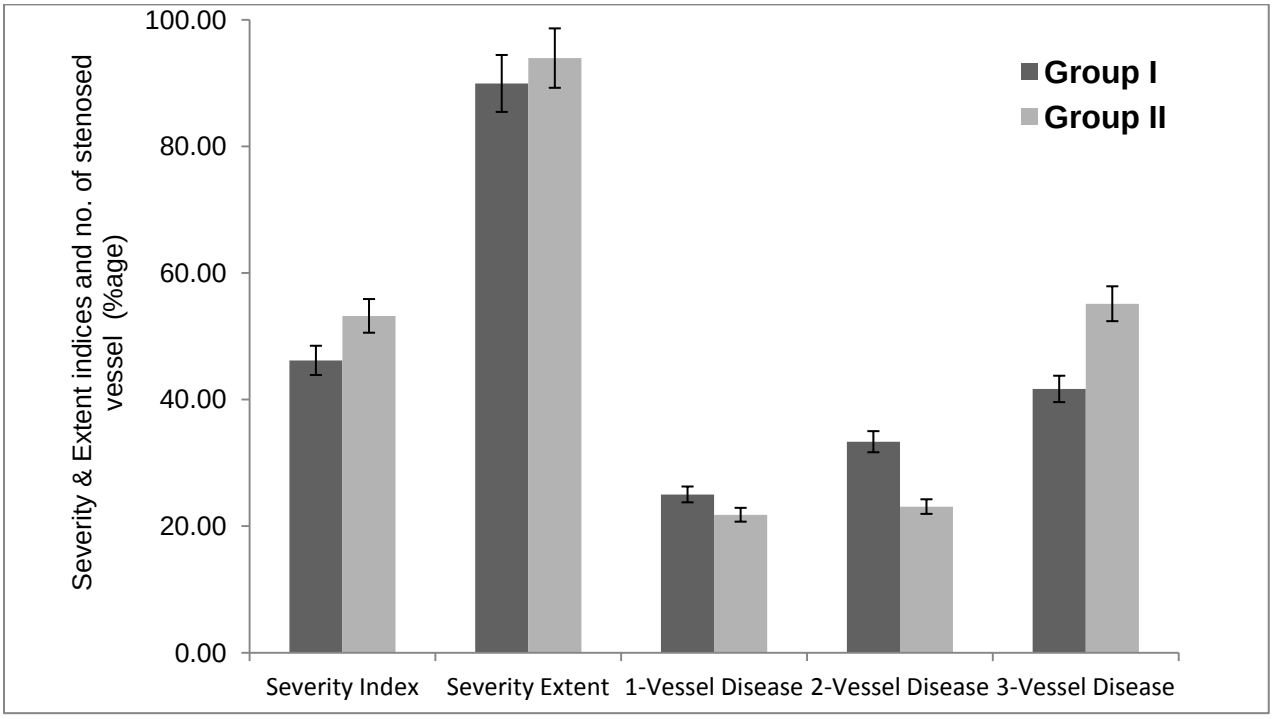
model 2, the analysis was additionally adjusted for new biohumoral factors thought to be important for the outcome (AGEs and hsCRP). All statistical analyses were made using the Sigma Stat 3.5 software. A two-tailed probability (*p*) level ≤0.05 was considered statistically significant.

RESULTS

Clinical characteristics of the study population

Table 1 represents baseline demographic, hemodynamic and biochemical characteristics of study subjects. Serum AGEs and hsCRP levels were found higher in CAD patients than healthy controls. Group II patients had more no. of multi-vessel diseases and high CAD severity and extent indices than group I patients (Fig. 1).

Figure 1: Angiographic characteristic of the study population



Serum AGEs and hsCRP levels in CAD patients

Both serum AGEs (arbitrary florescence units, AFU) and hsCRP (mg/l) levels were significantly higher in group II CAD patients than in group I (p<0.001) (Table 1). Serum AGEs levels correlated directly with hsCRP levels of CAD patients (Pearson's r=0.915, 0.919, both p<0.001), in GP I and II, respectively. Although some risk factors were not equally distributed across groups (Table 1), we adjusted these potential confounders with a logistic regression model. Differences in AGEs and hsCRP across groups found in the unadjusted analysis remained significant after such adjustments.

Table 1: Demographic and hemodynamic characteristic of subjects

Parameters	Control (n=40)	Group-I (n=60)	Group-II (n=65)	ANOVA F	*p* value
Age (yrs)	30.45±1.24	51.94±0.98	51.03±0.68	69.11	<0.001
Gender (M/F)	18/2	32/4	62/16		
BMI (kgm^{-2})	21.98±0.43	24.09±0.37	23.66±0.27	5.53	<0.001
SBP (mmHg)	125±0.94	144±1.14	145±0.64	47.14	<0.001
DBP (mmHg)	75±0.80	86±0.48	87±0.25	60.68	<0.001
TC (mg/dL)	170.90±2.05	206.74±1.80	203.68±0.88	56.30	<0.001
TG (mg/dL)	125.31±4.21	187.28±3.45	182.77±2.18	88.09	<0.001
HDL (mg/dL)	49.52±0.38	37.67±0.32	39.02±0.33	124.46	<0.001
LDL (mg/dL)	96.32±1.79	131.62±1.97	128.10±1.07	151.24	<0.001
FBS (mg/dL)	94.19±0.86	137.59±3.63	133.81±2.19	66.64	<0.001
HbA1c (%)	6.82±0.14	9.52±0.13	9.42±0.09	55.65	<0.001
hsCRP (mg/L)	3.04±0.14	4.85±0.15	5.23±0.10	31.73	<0.001
AGEs (AFU)	13.32±0.16	17.66±0.31	18.43±0.21	34.28	<0.001

Data presented as mean± SEM. Control = no CAD no risk factor, GP I = CAD with 3 risk factors (Hypertension + Dyslipidemia + Diabetes mellitus type-2), GP II = CAD with more than 3 risk factors. BMI = Body Mass Index, SBP = Systolic Blood Pressure, DBP = Diastolic Blood Pressure, TC = Total Cholesterol, TG = Triglycerides, HDL = High Density Lipoprotein, LDL = Low Density Lipoprotein, FBS = Fasting Blood Sugar, HbA1c = Glycated Hemoglobin, hsCRP = High Sensitivity C-Reactive Protein, AGEs = Advanced Glycation End Products

AGEs and hsCRP relate to QCA-derived CAD extent & severity and no. of diseased vessel

Significant differences existed in serum levels of AGEs and hsCRP among CAD patients with multiple cardiac risk factors (groups I, II) stratified for the number of diseased coronary arteries (p<0.001, Table 2). The number of diseased coronary arteries was closely related to serum levels of AGEs (Spearman's r=0.799, 0.863, p<0.001) and hsCRP (Spearman's r=0.833, 0.861, p<0.001) in groups I and II, respectively. In addition, serum AGEs and hsCRP levels were significantly correlated with QCA-derived severity index (Pearson's r=0.882, 0.825, both p<0.001) in GP I; (Pearson's r=0.822, 0.769, both p<0.001) in GP II and extent index (Pearson's r=0.946, 0.891, both p<0.001) in GP I; (Pearson's r=0.937, 0.835, both p <0.001) in GP II, respectively (Fig. 2), by taking control group (no significant CAD/no cardiac risk factors as in inclusion criteria) as 0-vessel disease.

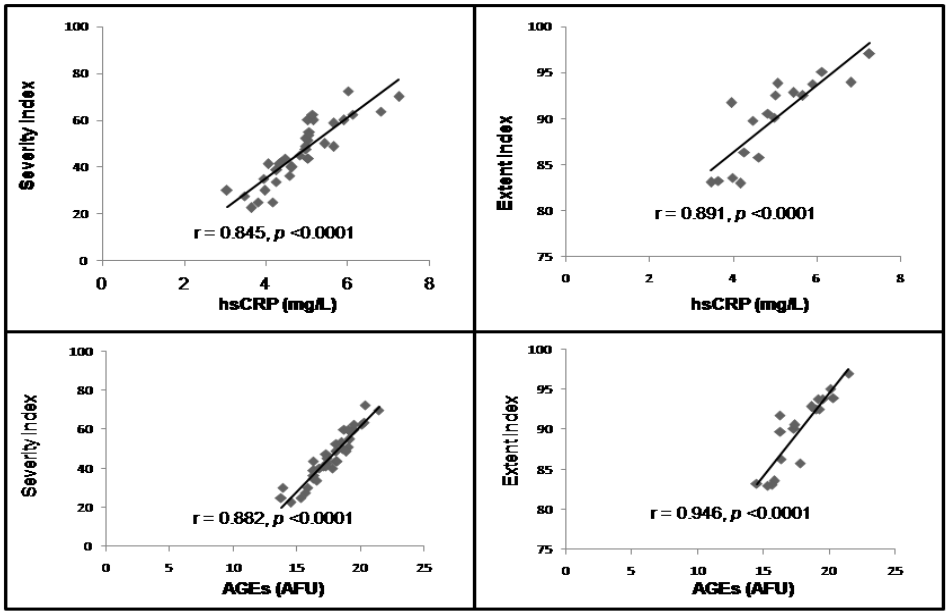
Table 2: Biomarkers level in patients stratified according to the number of diseased coronary arteries

Group	Variables	1-Vessel disease	2-Vessel disease	3-Vessel disease	ANOVA, F	*p* value
Group I	hsCRP	3.913±0.159	4.66±0.137	5.569±0.186	22.959	<0.001
	AGEs	15.496±0.442	17.247±0.233	19.299±0.239	41.954	<0.001
Group II	hsCRP	4.383±0.0904	4.811±0.0626	5.747±0.138	27.059	<0.001
	AGEs	16.124±0.179	17.579±0.179	19.725±0.194	78.104	<0.001

Data presented as mean ± SEM.

Significant correlations of AGEs and hsCRP with traditional risk factors like age (Pearson's r=0.514, 0.45, both p <0.001); SBP (Pearson's r=0.722, 0.62, both p<0.001); DBP (Pearson's r=0.144, 0.164, p=0.049, 0.025); HDL (Pearson's r = -0.203, -0.143, p=0.005, 0.05), FBS (Pearson's r=0.374, 0.447, both p<0.001) and HbA1c (Pearson's r=0.528, 0.577, both p<0.001), respectively, were also found in CAD patients.

Figure 2: Association between AGEs and hsCRP levels and QCA-derived extent and severity indices in group I (A) and group II (B) patients.



Multivariable regression analysis

Multivariable analysis, including traditional cardiovascular risk factors and biochemical measurements here performed, revealed that SBP and HDL were independently associated with QCA-derived CAD severity index; SBP was independently associated with QCA-derived CAD extent index in group I patients (Model 1, Table 3A). These factors, along with male gender, age, TC, and FBS, were also independent risk factors for 3-vessel disease in group I patients (Model 1, Table 3A). When AGEs and hsCRP were included in the multivariable analysis in model 2, they remained independently associated with CAD severity & extent index and 3-vessel disease. On the contrary, TC, HDL and FBS were not independent determinants of triple vessel disease in group I CAD patients (Table 3A).

Table 3A: Independent predictors of severity & extent indices (group I)

	Severity Index				Extent Index				3-Vessel Disease		
	β	t	*P*		β	t	*p*		OR	95% CI	*p*
					Model-1						
SBP	**1.598**	**0.837**	**<0.001**	**SBP**	**0.538**	**3.664**	**<0.001**	**Male**	**0.263**	**0.626-1.102**	**0.050**
HDL	**-1.329**	**-0.186**	**0.040**					**Age**	**1.792**	**1.176-2.731**	**0.007**

								SBP	5.542	1.153-16.644	0.033
								TC	1.213	1.037-1.419	0.016
								HDL	1.054	0.997-1.116	0.006
								FBS	1.052	0.999-1.107	0.050
					Model-2						
SBP	0.433	0.227	0.014	SBP	0.529	3.473	0.003	Male	0.257	0.792-0.830	0.045
HDL	-0.871	-0.122	0.019	hsCRP	0.582	0.494	0.041	Age	1.834	1.212-2.776	0.004
hsCRP	4.986	3.642	<0.001	AGEs	0.461	1.509	0.007	SBP	2.989	1.347-6.634	0.007
AGEs	5.302	4.055	<0.001					hsCRP	2.947	1.013-2.206	0.011
								AGEs	3.963	2.211-5.217	0.011

OR = Odds Ratio, CI = Confidence Interval

In group II patients, multivariable analysis, including traditional cardiovascular risk factors and biochemical measurements here performed, revealed that age, SBP, TG and HbA1c were independently associated with QCA-derived CAD severity index; age, SBP and HbA1c were independently associated with QCA-derived CAD extent index; age, SBP, TC, TG, HDL and LDL, were also independent risk factors for 3-vessel disease (Model 1), respectively (Table 3B). When AGEs and hsCRP were included in the multivariable analysis in model 2, they remained independently associated with CAD severity & extent index and 3-vessel disease. On the contrary, TG and HbA1c in case of CAD severity index; HbA1c in case of CAD extent index; and TC, TG, HDL and LDL in case of triple of vessel disease, were **not** independent determinant in group II CAD patients in model 2 (Table 3B).

Table 3B: Independent predictors of severity & extent indices (group-II)

	Severity Index				Extent Index				3-Vessel Disease		
	β	T	*P*		β	T	*P*		OR	95% CI	*P*
					Model-1						
Age	0.908	4.311	<0.001	Age	0.341	4.804	<0.001	Age	1.523	1.254-1.849	<0.001
SBP	1.399	6.867	<0.001	SBP	0.128	2.081	0.047	SBP	4.676	1.746-12.521	0.002
TG	0.0968	2.491	0.015	HbA_1c	0.992	2.515	0.018	TC	0.854	0.744-0.981	0.025
HbA_1c	2.483	8.521	0.005					TG	1.048	1.006-1.092	0.026
								HDL	0.788	0.641-0.969	0.024
								LDL	1.176	1.031-1.342	0.016
					Model-2						
Age	0.437	3.368	0.002	Age	0.234	3.017	0.001	Age	1.330	0.970-1.825	0.007
SBP	0.578	6.729	<0.001	hsCRP	0.618	1.805	0.046	SBP	4.360	1.756-10.824	0.002
hsCRP	3.937	7.004	0.050	AGEs	0.886	2.048	<0.001	hsCRP	1.116	2.378-3.552	0.002
AGEs	5.057	11.832	<0.001					AGEs	1.516	0.982-2.340	<0.001

OR = Odds Ratio, CI = Confidence Interval

DISCUSSION

Substantial evidence supports a role of the AGEs and hsCRP in the development of atherosclerosis, and CAD complications. Present study addressed the relationship between AGEs and hsCRP levels with CAD, assessed visually and by QCA, in CAD patients with multiple cardiac risk factors. Evidence supports the role of AGEs in the development and/or pathogenesis of CAD (Kanauchi *et al.,* 2001). Previous studies have demonstrated that AGEs induce oxidative stress in the vessel wall (Liu *et al.,* 2006; Pu *et al.,* 2006) enhances pro-inflammatory endothelial response (Ehlermann *et al.,* 2006), and promotes proliferation and migration of vascular smooth muscle cells (Hattori *et al.,* 2002), and thereby is associated with accelerated atherosclerosis.

Presented here is the first study addressing the association between AGEs and hsCRP levels ($p<0.001$) and with CAD severity and extent ($p<0.001$), in patients with multiple risk factors, more specifically in Indian population. These results support the hypothesis that the increased *in-vivo* synthesis of glycation products aggravates vascular inflammation, resulting in more severe CAD and associated complications (Peepa *et al.,* 2002). Our results are consistent with previous findings that reported serum AGEs levels were higher in diabetic, dyslipidemic, hypertensive and metabolic syndrome patients with vascular complications than in those without (Pu *et al.,* 2006; Holtzman *et al.,* 2008 ;Fornengo et al., 2006). In the present study, AGEs level was found to be an independent predictor of CAD (severity and extent indices) and number of stenosed vessels in CAD patients with multiple risk factors. Serum AGEs level was an independent predictor in patients of CAD with hypertension, diabetes and dyslipidemia (group I), and risk factors more than three (group II), all $p<0.001$, respectively. In combination of other risk factors as the level of AGEs increases the severity and extent of CAD too increases. In addition, AGEs was also an independent risk factor for number of vessel disease in both groups (I & II) of CAD patients. Every one unit rise in level of AGEs markedly increase the risk of stenosis for triple vessel disease [odds ratio (OR)=3.963] in group I and [OR=1.516] in group II, respectively.

The inconsistent level of AGEs may be due to the combined effects of small number of patients in each group and drugs used by patients like antioxidants (pyridoxine, alpha lipoic

acid), as these drugs inhibits post-Amadori formation of advanced glycation end-products (AGEs) and formation of advanced lipoxidation end-products (ALEs) on proteins during lipid peroxidation reactions (Metz *et al.,* 2003).

We observed a significant correlation of serum AGEs level with blood sugar, HbA1c, SBP, and HDL concentration (r=0.374, 0.528, 0.722, 0.144, -0.203, all $p \leq 0.05$), respectively. Therefore, these results indicate that determination of serum AGEs level appears to be a useful marker for predicting the presence of CAD and also a valuable adjunct to other variables in reflecting short-term glycemic, blood pressure and lipids control. Moreover, the positive correlation indicates increase in AGEs level parallel increase the blood pressure lipids and sugar level. The current study also revealed that serum level of AGEs ≥15 (AFU) was associated with approximately 2- to 4-fold increase in risk of 3-vessel CAD in study population when compared with control AGEs level≤15 (AFU).

Atherosclerosis is a long-term and chronic inflammatory process (Ross 1999; Muhlestein *et al.,* 2006) and CRP has been shown to be associated with atherosclerosis and acute cardiovascular events (Wilson *et al.,* 2006; Monakier *et al.,* 2004; Berk *et al.,* 1990). Researchers showed that patients at intermediate or high risk of CAD may benefit from measurement of hsCRP with regard to individual risk prediction [Monakier *et al.,* 2004; Pfutzner *et al.,* 2006; Ridker *et al.,* 2003; Koenig *et al.,* 1999). In the present study, hsCRP was the independent predictor of CAD severity and extent indices in both groups (both $p<0.001$). The present study also showed that hsCRP was an independent risk factor for 3-vessel disease in group I (OR=2.947, p=0.011) and in group II (OR=1.116, p=0.002) patients, respectively.

The inconsistency in results may be due to the less number of patients, their distribution in each group and use of anti-inflammatory and other drugs. Aspirin and other cyclo-oxygenase (COX) inhibitors act by direct anti-inflammatory action and through platelet aggregation inhibition (Kennon *et al.,* 2001), statins, fibrates and their combinations indirectly (Muhlestein *et al.,* 2006) lowers the CRP levels prescribed to the several patients receiving for last several months before going to angiography and entering in study. Overall, the current study revealed that serum level of hsCRP≤5mg/L was associated with approximately 1- to 3-fold increase in risk of 3-vessel CAD in study population when compared with hsCRP level<5mg/L in non-CAD

patients. These results indicate that serum levels of hsCRP reflect inflammatory status, which merits hsCRP to be an independent biomarker for predicting CAD.

Our study indicates that AGEs and hsCRP are capable of reflecting the clinical manifestations of CAD in patients with multiple cardiac risk factors. These results substantiate the concept that AGEs (Kanauchi *et al.*, 2001) and inflammatory factors (hsCRP) (Arroyo-Espliguero *et al.*, 2004; Manginas *et al.*, 2005) play a crucial role in the pathophysiology of CAD, and are useful biomarkers of instability and adverse prognosis.

Hypertension (SBP) was also the independent predictor of both CAD severity and extent in all the groups. Several clinical trials demonstrate that, SBP plays a larger role than DBP in predicting adverse CV outcomes, particularly in elderly patients (Staessen *et al.*, 1997). SBP was an independent predictor of CAD severity and extent in group I ($p<0.001$, 0.003) and severity in group II ($p<0.001$). It was also an independent predictor for 3-vessel disease, in both group I and group II patients.

In the present study, most of traditional risk factors (including, HDL-C, LDL-C, total cholesterol, triglyceride, body mass index, FBS and HbA1c) could not enter the model-2 of multiple regression analysis for predicting CAD severity and extent, and no. of vessel disease, except those entered (HDL), suggesting that these traditional risk factors were not only and real risk factors for CAD development. However, we do believe that the impact of these risk factors cannot be excluded because lipids may exert detrimental effects depending on how much they become glycated and oxidized. Glycated and/or oxidized LDL have demonstrated adverse effects on vascular cell viability, lipid accumulation, growth factor expression and intracellular oxidative stress (Jerkins *et al.*, 2004). Modification of HDL by glycation and oxidation may ameliorate efficacy of its vasoprotective functions (Pu *et al.*, 2006; Jerkins *et al.*, 2004). These are not easy to determine at present. In addition, majority of the patients in our study received medication of statins, and these lipids are highly sensitive to statin treatment, and some were receiving beta blockers which may adversely affect serum cholesterol (Lakshman *et al.*, 1999). This may partly explain why these were not entered the model. Blood sugar and HbA1c was also not an independent predictor of CAD severity and no. of vessel disease. Our results are supported by the previous findings that revealed elevated fasting glucose levels were predictive of future all-cause and CV mortality and believed to play a role in the pathogenesis of CAD;

however, the cut-off level beyond which glycemia become detrimental remains controversial (Nigam *et al.,* 2007) and also, may be due to the use of several anti-diabetic drugs (like sulfonyl ureas, biguanides, meglitinides and thiazolidinediones) (Lu *et al.,* 2009) by the patients for several months.

LIMITATIONS

We recognize several limitations in our study. Firstly, the classification of significant CAD as the angiographic percent stenosis≥70% at the site of coronary artery lesions is to some extent arbitrary. However, within the range of angiographically significant CAD including lesions of ≥70%, this criterion of stenosis severity has been found to have pathophysiologic significance, has relevance to commonly applied angiographic standards, and is widely accepted in clinical practice (Scanlon *et al.,* 1999). QCA has to be intended as an approximate (albeit operator-independent) estimate of coronary atherosclerosis, since angiography can admittedly only detect the lumen impingement due to atherosclerosis and not the intimal plaque component expanding towards the outside of the vessel in the process known as positive remodeling (De Caterina *et al.,* 2006). Our data was only based on coronary angiography findings, by which less stenotic plaques may not be detected. Therefore, results did not include all atherosclerotic lesions in coronary arteries. Secondly, this is in fact a small size, prospective, case control study, thereby allowing to detect associations and to formulate risk predictions to some extent only. But the predictive value of AGEs and hsCRP certainly at this point needs to be assessed during a long-term follow-up in large size population. Further studies are required to include large sample size to ascertain the differences in biomarkers and biochemical measurements between different groups of patients with CAD and controls. As follow-up of the patients has not carried out and therefore, our logistic regression model of CAD prediction could not test the long term patient's outcome and disease progression. Thirdly, hsCRP was the only inflammatory factor measured in this study. Whether other "noxious" pro-atherosclerosis inflammatory factors, such as TNF - α , interleukin-6, play a role in the development of CAD in this cohort remains to be answered. As suggested in some studies, a cluster of "harmful" interleukins exert synergistic and convergent effects on cardiovascular diseases (Fisman *et al.,* 2003). Determination of interleukin helps understand the mechanisms and disease progression, and thereby provides potential guidance to

control atherosclerosis. Among these possible factors, soluble receptor of AGEs and other factors associated with AGE-receptor system may also requires further investigation.

CONCLUSION

Serum AGES and hsCRP levels along with other traditional risk factors were closely associated with the number of diseased coronary arteries, as well as with QCA-assessed angiographic CAD severity and extent indices, and were independent correlates, in the sample population. Though large sample size was required for making any risk prediction, we hereby suggest risk prediction on the basis of sample size of 125 patients, that may not provide true value of risk prediction but, may act as root source for future studies in Indian population. Overall, in CAD patients with at least three co-morbidities, 'hsCRP'≥4.8 mg/L and 'AGEs'≥17 AFU were found to increase 1 to 3-times and 1 to 4-times risk of TVD, respectively. These two risk factors along with traditional risk factors act independent predictors for CAD extent and severity. Thus, AGEs and hsCRP may act potentially useful markers, for predicting CAD severity & extent and 3-vessel disease in patients with multiple cardiac risk factors.

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