

# LEVEL OF HIGH-SENSITIVITY C-REACTIVE PROTEIN IN SAUDI PATIENTS WITH CHRONIC STABLE CORONARY ARTERY DISEASE

Syed Shahid Habib

Department of Physiology, College of Medicine, King Saud University, Riyadh, Kingdom of Saudi Arabia

**Background:** Inflammatory biomarker high-sensitivity C-reactive protein (hsCRP) is an independent predictor of future cardiovascular events and it predicts risk of incident hypertension and diabetes. The aim of this study was to determine the serum levels of the circulating acute-phase reactant high-sensitivity C-reactive protein (hsCRP) in Saudi patients with chronic stable Coronary Artery Disease (CAD). **Methods:** This cross sectional study was conducted in the Department of Physiology and Department of Cardiology, College of Medicine and King Khalid University Hospital, King Saud University, Riyadh between August 2006 and December 2007. One hundred and seven individuals with chronic stable CAD and 33 healthy, age and BMI-matched individuals were studied. Overnight fasting blood samples were collected, and analyzed for total cholesterol (TC), Triglycerides (TG), Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL) and hsCRP in patients with chronic stable CAD. **Results:** TC (Control  $4.41 \pm 0.57$  vs CAD  $4.28 \pm 1.40$ ,  $p=0.8394$ ) and LDL levels (Control  $2.70 \pm 0.52$  vs CAD  $2.71 \pm 1.20$ ,  $p=0.7963$ ) did not differ significantly between the two groups. While there were significant differences in TG (Control  $1.13 \pm 0.47$  vs CAD  $1.84 \pm 1.10$ ,  $p=0.0135$ ) and HDL levels (Control  $1.06 \pm 0.30$  vs CAD  $0.71 \pm 0.25$ ,  $p=0.0000$ ). hsCRP levels were significantly higher in patients with CAD ( $5.0 \pm 4.4$ ) compared to healthy individuals ( $2.7 \pm 2.7$ ,  $p=0.0166$ ). Frequency of low risk levels was significantly higher in Controls than CAD patients (24.2% vs 8.4%,  $p=0.0332$ ) and vice versa for high risk levels (24.2% vs 51.4%,  $p=0.0110$ ). At average risk levels frequency did not differ significantly (51.5% vs 40.1%,  $p=0.3429$ ) between control and CAD groups. **Conclusion:** Saudi patients with stable chronic CAD have higher hsCRP levels compared to healthy individuals. Moreover the prevalence of undesirable risk levels of hsCRP is also higher in CAD patients. **Keywords:** coronary artery disease, High-sensitivity C-reactive protein, inflammation, cardiovascular risk, prevalence, risk levels

## INTRODUCTION

A large body of evidence suggests that inflammation plays a key role in the pathogenesis of atherosclerosis. The chronic inflammatory process can develop to an acute clinical event by the induction of plaque rupture and therefore cause acute coronary syndromes.<sup>1</sup> More than 20 large prospective trials have shown that the inflammatory biomarker high-sensitivity C-reactive protein (hsCRP) is an independent predictor of future cardiovascular events plus it predicts risk of incident hypertension and diabetes.<sup>2</sup> Several studies from both the United States and Europe indicate that elevated levels of hsCRP among apparently healthy men and women are a strong predictor of future cardiovascular events.<sup>3-6</sup> A long-term predictive value of elevated hsCRP levels has been found in patients with documented coronary artery disease and angina<sup>7,8</sup> and in individuals with multiple risk factors.<sup>9</sup> hsCRP not only predicts first myocardial infarction but also recurrent events.<sup>10-12</sup> In most of these studies, effect of hsCRP on vascular risk remained highly significant after adjustment for traditional risk factors typically used in global risk-assessment programs.<sup>5</sup>

The aim of this study was to determine the serum levels of the circulating hsCRP and frequency of different risk levels distribution according to American Medical Association International Guidelines, in Saudi patients with chronic stable coronary artery disease (CAD) and to compare it with matched healthy subjects.

## MATERIAL AND METHODS

This cross sectional study was conducted at the department of Physiology and department of Cardiology of College of Medicine & King Khalid University Hospital, King Saud University, Riyadh between August 2006 and December 2007. It was funded by College of Medicine Research Centre (CMRC). The study protocol was approved by the Research Ethics Committee of CMRC.

The individuals selected were informed about the details of the study and those patients who agreed to participate signed the consent form which was typed both in English and Arabic language.

A clinical record of each individual including personal data, demographic data, family history and result of the coronary angiography was filled in a predesigned proforma. Inclusion criteria included adult Saudi patients of any sex with chronic stable CAD who had attacks of Angina or Myocardial infarction. The individuals with a history of myocardial infarction or unstable angina during the previous four weeks as well as subjects with a history of percutaneous transluminal coronary angioplasty were excluded. The diagnosis of myocardial infarction required the presence of at least 2 of these criteria (1) A history of characteristic prolonged ( $\geq 30$  min) pain or discomfort, (2) Creatine kinase (CK) elevation exceeding twice the upper limit of normal (or CK-MB  $\geq 50\%$  of total CK), and (3) Presence of new Q waves or new abnormal ST-T features.

Unstable angina was defined as pain at rest with at least 2 episodes during the previous 48 h, at least one of which lasted  $\geq 20$  min, or ST-segment deviations that were diagnostic of myocardial ischemia during anginal attack, with no elevation of MB fraction of CK or lactate dehydrogenase on admission to the hospital.<sup>13</sup> Furthermore, individuals with concomitant systemic diseases (thyroid disorders, acute infections, stroke, diabetic ketoacidosis, non-ketotic hyperosmolar diabetes rheumatic diseases, chronic liver diseases, renal disorders, cancer and sepsis) and subjects who were critically ill or with ongoing or recent (<1 month) infectious diseases as well as patients with surgical procedure in last 3 months were excluded. The results of patients with hsCRP values >10 mg/L was discarded and were re evaluated after 2–3 weeks. We followed the guidelines of the American Heart Association for measurement, evaluation and expression of hsCRP.<sup>14</sup>

As per selection criteria 107 patients were finally selected from a group of one hundred and thirty six subjects. Overnight fasting blood samples were collected; serum was separated and stored at -80 °C.

Fasting Venous blood samples were analyzed for Lipids comprising total cholesterol (TC), Triglycerides (TG), Low density Lipoprotein (LDL) and High density lipoprotein (HDL) and hsCRP. TC, TG, LDL and HDL were analyzed by enzymatic colorimetric method. The instrument used was autoanalyser Dimension (USA) and the kits were also provided by the same company. hsCRP was measured with a high-sensitivity latex-enhanced turbidimetric assay with Quantex CRP ultra sensitive kits supplied by BIODATA Spain. CRP Reagent is a suspension of polystyrene latex particles of uniform size coated with rabbit IgG anti human CRP. When a sample containing CRP is mixed with the reagent, a clear agglutination occurs, which can be measured by turbidimetry. Results are expressed in  $\mu\text{g/dl}$  based on international reference material for measurement of 14 human serum proteins (CRM 470). One important attribute of C-reactive protein is its stability over the time and the availability of automated methods by which to measure it. The newest assays are very sensitive and provide measurement of C-reactive protein at levels substantially below those levels measured by traditional methods. The kit had a working range from 0.10 to 20.0 mg/L. The autoanalyser used was Hitachi 911, manufactured by ROCHE diagnostics, USA.

The data was analyzed by computer software program Statistical Package for Social Sciences (SPSS version 10, Chicago). Descriptive characteristics and lipid profile of the study patients were calculated as Mean $\pm$ SD (Standard Deviation) for continuous variables and as percentages for categorical variables. Student's *t*-test was used for comparison between studied groups. A *p*-value of <0.05 was considered as statistically significant. The relative percentage distribution of individuals in different

groups with desirable and high risk levels of hsCRP was determined. Categorical variables were compared between various groups using Chi square test. Spearman's correlation coefficient was determined between hsCRP and clinical characteristics in CAD patients.

## RESULTS

Clinical characteristics, lipid profile and hsCRP levels of Control and all CAD patients are shown in Table-1. There were non significant differences between age, BMI and Blood pressure between Control and CAD subjects. TC and LDL levels did not differ significantly between the two groups while TG were significantly higher in CAD patients compared to Control subjects. Moreover HDL levels were significantly lower in CAD subjects versus Controls (Table-1). hsCRP levels were significantly higher in patients with CAD (5.0 $\pm$ 4.4) compared to healthy individuals (2.7 $\pm$ 2.7, *p*=0.0166). Frequency of low risk levels was significantly higher in Controls than CAD patients (24.2 % vs 8.4 %, *p*=0.0332) and vice versa for high risk levels (24.2% vs 51.4%, *p*=0.011). At average risk levels frequency did not differ significantly (51.5% vs 40.1%, *p*=0.3429) (Table-2).

No significant correlation was observed of hsCRP with age (*r*=0.099, *p*=0.670), BMI (*r*=0.201, *p*=0.086), SBP (*r*=0.421, *p*=0.058) and DBP (*r*=0.269, *p*=0.239) in CAD patients. However there was a significant positive correlation of hsCRP with pulse (*r*=0.506, *p*=0.019) and negative correlation with ejection fraction (*r*=-0.283, *p*=0.014) in these patients.

**Table-1: Clinical and biological data of Control and all CAD patients. Values are expressed as Mean $\pm$ SD**

	Control	All CAD	<i>p</i> -value
N	33	107	
Gender M/F	21/12	75/32	
Age	53.70 $\pm$ 8.62	57.56 $\pm$ 11.94	0.2883
BMI	26.41 $\pm$ 3.58	28.18 $\pm$ 6.29	0.2646
BP Systolic	125.31 $\pm$ 15.48	132.33 $\pm$ 20.77	0.1352
BP Diastolic	75.67 $\pm$ 10.91	76.87 $\pm$ 16.00	0.1948
TC mmol/L	4.41 $\pm$ 0.57	4.28 $\pm$ 1.40	0.8394
TG mmol/L	1.13 $\pm$ 0.47	1.84 $\pm$ 1.10	0.0135
LDL mmol/L	2.70 $\pm$ 0.52	2.71 $\pm$ 1.20	0.7963
HDL mmol/L	1.06 $\pm$ 0.30	0.71 $\pm$ 0.25	0.0000
CRP mg/L	2.7 $\pm$ 2.72	5.0 $\pm$ 4.41	0.0166
CRP Median	1.73	3.09	

**Table-2: Percentage distribution of different risk categories of Control and CAD patients.**

Risk Categories	Standard Risk Levels mg/L*	Control No. (%)	CAD No. (%)	<i>p</i> -value	Odds ratio	95% Confidence limits
Low	<1.0	8 (24.2)	9 (8.4)	0.0332	3.48	1.08-11.18
Average	1.0 to 3.0	17 (51.5)	43 (40.1)	0.3429	1.58	0.67-3.72
High	>3.0	8 (24.2)	55 (51.4)	0.0103	0.302	0.11-0.78

\*American Medical Association Guidelines.<sup>15</sup>

## DISCUSSION

We observed that hsCRP levels were significantly higher in patients with CAD compared to healthy individuals. Moreover the prevalence of undesirable risk levels of hsCRP is also higher in CAD patients in the high risk ranges and the difference was significant in low risk ranges, but there was non significant difference in prevalence of average risk range between CAD and controls. A possible reason is that we do not have risk levels defined for this population and large scale studies are needed in this regard to get the actual risk levels for this population and this has been suggested in guidelines also.<sup>14</sup>

Serum hs-CRP been reported by other colleagues to be an independent predictor of angiographically defined CAD in an Iranian population. They suggested that measurement of the serum hs-CRP level may improve risk stratification among patients suspected of having CAD. The strong correlations of serum hs-CRP with LDL and smoking may be due to the putative pro-inflammatory effects of these two parameters.<sup>15</sup>

The CRP measurement has a lot of advantages. Firstly it is a stable compound and secondly it can be measured at any time of the day without regards to biological clock. In contrast to results for cytokines such as IL-6, no circadian variation appears to exist for hsCRP. Thus, clinical testing for hsCRP can be accomplished without regard for time of day.<sup>16</sup>

In current strategies of global risk assessment, lipid testing is the only blood test routinely recommended. However, hsCRP evaluation may have the potential to improve cardiovascular risk prediction models when used as in addition to traditional lipid profiles.<sup>3,10</sup>

Baseline CRP levels in the subgroup of patients with AMI ( $6.49 \pm 2.28$  mg/L) were significantly higher than levels in patients with stable CAD ( $4.35 \pm 2.6$  mg/L). We studied hsCRP levels in metabolically stable CAD patients and our values are comparable to this study.<sup>17</sup>

In our study although CRP seems to be an independent discriminator between the patients with CAD and the control group, there was considerable overlap in CRP concentrations between the two populations in average risk ranges. Similar findings have been reported by Haidari *et al.*<sup>18</sup>

The CRP level is quite efficient for separation of patients from controls. Therefore keeping in mind the lack of specificity, the CRP level may be a useful tool in the diagnosis of coronary heart disease.<sup>19</sup>

## CONCLUSION

Saudi patients with stable chronic CAD have higher hsCRP levels compared to healthy individuals. Moreover the prevalence of undesirable risk levels of hsCRP is also higher in CAD patients particularly in higher risk ranges.

The hsCRP has a strong association with stable CAD and the measurement of CRP may improve coronary risk assessment in Saudi patients with CAD.

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**Address for correspondence:**

**Dr Syed Shahid Habib**, Assistant Professor, Department of Physiology (29), College of Medicine, PO Box 2925, King Saud University, Riyadh 11461, Kingdom of Saudi Arabia. Tel: +966-1-4671604, Fax: +966-1-4672567  
**Email:** shahidhabib44@hotmail.com