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Hs-CRP - A Potential Marker for Coronary Heart Disease

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ABSTRACT

Inflammatory reactions in coronary plaques play an important role in the pathogenesis of acute atherothrombotic events; inflammation elsewhere is also associated with both atherogenesis generally and its thrombotic complications. The aim of study was to investigate the predictive value of Hs-CRP (high sensitivity C-reactive protein) in coronary heart disease (CHD).The study population contained 200 subjects divided into two groups, 150 patients with CHD and 50, age and sex matched healthy control subjects. Hs-CRP is a marker of inflammation in coronary heart disease. The level of CRP in the serum samples was estimated by a high sensitivity immunoturbidometric assay. Hs-CRP, total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL) levels, were found significantly high in coronary heart disease patients as compared to healthy subjects but significant decrease in high density lipoprotein cholesterol (HDL-C) in CHD patients as compared to healthy controls. In conclusion, our study shows a significant increase in Hs-CRP, TC, TG, LDL-C and VLDL-C in the circulation of coronary heart disease patients. A significant decrease in levels of HDL-C was observed only in CHD patients. The concentration of Hs-CRP (inflammatory marker) is increased in coronary heart disease subjects. Therefore these biomarkers may be useful in diagnosis of coronary heart disease.

Key Words: Coronary Heart Disease, Inflammatory Marker, Hs-CRP (High Sensitivity C - reactive protein)

INTRODUCTION

Inflammation plays a role in the development of atherosclerosis and coronary heart disease (Lind, 2003). Elevated markers of inflammation, in particular CRP, are associated with an increased risk of future cardiovascular events in healthy subjects, in patients with stable or unstable coronary artery disease and acute myocardial infarction (Buffon *et al.*, 2002; Zairis *et al.*, 2002). The most common cause of coronary heart disease is atherosclerosis with erosion or rupture of a plaque causing transient, partial or complete arterial occlusion. Heart cannot continue to function without adequate blood flow, and if it is severely compromised, death is inevitable. Several risk factors for coronary heart disease have been well documented, including hypertension, hyperlipidemia, diabetes, a positive family history of CHD, smoking, obesity and physical inactivity (Kasap *et al.*, 2007). C-reactive protein (CRP) is the prototype acute-phase protein primarily synthesized in the liver and its release is stimulated by interleukin 6 (IL-6) and other pro inflammatory cytokines (Daniel *et al.*, 2003). Composed of five 23 kDa subunits, C-reactive protein (CRP) is a hepatically derived pentraxin that plays a key role in the innate immune response. CRP has a long plasma half-life and is now understood to be a mediator as well as a marker of atherothrombotic disease. High sensitive CRP (Hs-CRP) has been shown to have prognostic value in patients with acute coronary syndromes; however, the most promising use of Hs-CRP has been in the primary prevention setting. Hs-C-reactive protein not only may be a marker of low grade chronic systemic inflammation but also may be directly involved in atherosclerosis (Ridker *et al.*, 2003). It can amplify the anti-inflammatory response through complement activation, tissue damage, and activation of endothelial cells (Libby *et al.*, 2002). In the present communication, we assessed the high sensitivity C-reactive protein in patients with coronary heart disease with age and sex matched healthy subjects.

MATERIALS AND METHODS

The population for study consisted of 200 subjects divided into two groups, 150 subjects (mean age 31 to 70 years) had coronary heart disease and the other 50 subjects were age and sex matched healthy control. Study subjects were taken from the outdoor and indoor department of medicine of our institute. Healthy control subjects were selected from the institution. The diagnosis of coronary heart disease was confirmed by clinical presentation and other investigations like characteristic electrocardiogram (ECG) changes, positive trademill test and positive echocardiographic findings. The patients who had total cholesterol level of >250 mg/dL or triglycerides concentration >200 mg/dL, or receiving lipid lowering drugs were defined as having hyperlipidemia.

Exclusion Criteria

Confounding factors which could interfere in the biochemical analyses of study subjects and alter the results are smoking, diabetes, active inflammatory diseases, nutritional deficiencies, estrogen therapy, and collagen disease arthritis. Patients with these diseases were excluded from the study. The same exclusion criteria utilized for cases were applied for control selection. All the above exclusion factors were confirmed from the patient's personal physician report and history.

Collection and Analysis of sample

Blood samples were drawn from patients and controls. Five ml blood was collected in plain vials (without any anticoagulant) for estimation of serum lipids (total cholesterol, triglycerides, and high density lipoprotein cholesterol), serum high sensitivity C-reactive protein.

Estimation of Serum High Sensitivity C - Reactive Protein (Hs-CRP)

As a measure of high sensitivity C-reactive protein was estimated by immunoturbidometry method (Otsuji *et al.* 1982). (The detection range for Hs-CRP is ≤ 5 mg/L). CRP causes agglutination of the latex particles coated with anti-human CRP. The agglutination of the latex particles is proportional to the CRP concentration and can be measured by turbidimetry.

Lipid Analysis

Lipid profile was estimated by commercially available kits. Serum total cholesterol (TC) estimated by enzymatic Cholesterol Oxidase Peroxidase (CHOD-POD), end point method (Allain *et al.*, 1974). Serum triglyceride (TG) estimated by enzymatic Glycero Phosphate Oxidase Peroxidase GPO/POD, endpoint method (Bucolo *et al.* 1973) at 510 nm. Serum high density lipoprotein cholesterol (HDL-C) estimated by Phosphotungstic Acid, End Point method (Assmann 1983) at 510 nm. Serum very low density cholesterol (VLDL-C) and low density lipoprotein cholesterol (LDL-C) were calculated from the Friedwald's formula (Friedwald *et al.*, 1972). All parameters were judged spectrophotometrically.

RESULTS

The sex distribution, mean age and lipid parameters are shown in table 1. The mean age was 44.02±12.8 years in control subjects and 53.73 ± 9.27 years in CHD subjects. Comparison of serum total cholesterol between healthy controls and CHD subjects showed significant differences (P = <0.0001). Mean value of serum total cholesterol (TC) in healthy control was 169.0 ± 22.2 mg/dl and in CHD subjects was 247.0 ± 45.8 mg/dl. Comparison of serum triglyceride between healthy controls and CHD subjects showed significant differences (P =<0.0001). Mean value of serum triglyceride (TG) in healthy controls was 113.0 ± 22.8 mg/dl and in CHD subjects was 192.0 ± 52.5 mg/dl. HDL-C level was significantly lower in males of healthy controls (t = 5.02; P = 0.005) and CHD subjects (P = 0.002) as compared to females of the respective groups. Comparison of mean serum high density lipoprotein cholesterol in between healthy controls and CHD subjects was highly significant (P =< 0.001). Comparison of mean serum very low density lipoprotein cholesterol in between healthy controls and CHD subjects was significant (P = <0.001). Mean value of serum very low density lipoprotein cholesterol (VLDL-C) in healthy controls was 22.6 ± 4.5 mg/dl and CHD subjects was 38.4 ± 10.5 mg/dl. The increase in mean serum low density lipoprotein cholesterol level in CHD subjects was statistically significant (P = <0.0001) as compared to in healthy controls as shown in Table 1. Hs-CRP level significantly increased (P = <0.001) in CHD subjects as compared to healthy controls. Mean levels of Hs-CRP in healthy controls and CHD subjects were 0.93 ± 0.35 mg/L and 1.7 ± 0.75 mg/L respectively (Table 2, Fig. 1). These observations imply that a subclinical inflammatory reaction has a role in atherosclerosis in coronary heart disease as shown in Table 2.

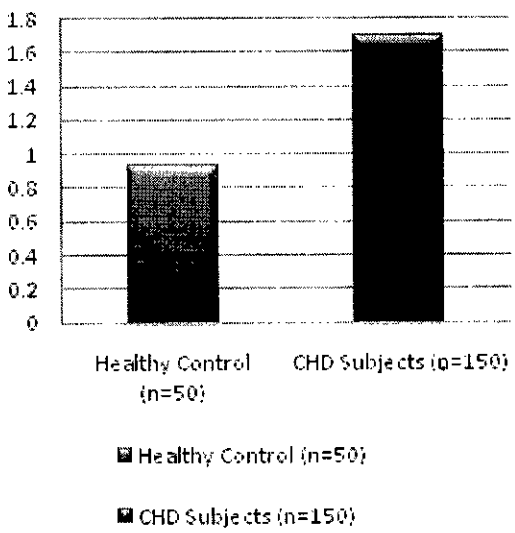

Fig. 1: Mean values of Serum High Sensitivity C-reactive protein levels in healthy control and Coronary Heart Disease subjects

DISCUSSION

Inflammation plays an important role in the development of coronary heart disease. Inflammation of coronary arteries is likely an important component of changes in vessel wall morphology (Alexander 1994, Vander Wai *et al.*, 1994). It has been proposed that inflammation of arteries results in an increased production of cytokines, especially interleukin 6, and activation of clotting factors, increased platelet aggregation, and smooth muscle cell proliferation (Pepys and Balt 1983). Vahdat *et al.*, (2007) found that elevated Hs-CRP was significantly correlated with electrocardiogram defined coronary artery disease. A significant difference has been observed regarding the value of Hs-CRP in CHD subjects as compared to normal healthy control subjects in our study which reveals that there is an association between Hs-CRP and CHD. Our results are in accordance with the previous result (Pasupathi *et al.*, 2009).

Table 1: Mean Age and levels of lipid parameter in coronary heart disease (CHD) patients and control subjects

Clinical Character	CHD Subjects (n = 150)	Control Subjects (n =50)	P
Sex (M/F)	94/56	30/20	-
Age (years)	53.73 ± 9.27	44.02 ± 12.8	-
Total cholesterol (mg/dl)	241.24 ± 45.83	169.6 ± 22.24	<.001
Triglycerides (mg/dl)	191.15 ± 52.54	113.32 ± 22.82	<.001
HDL-Cholesterol (mg/dl)	40.99 ± 8.30	51.86 ± 7.08	<.001
LDL-Cholesterol (mg/dl)	167.69 ± 39.5	97.03 ± 21.51	<.001
VLDL-Cholesterol (mg/dl)	38.43 ± 10.51	22.67 ± 4.56	<.001

Table 2: Mean values of Serum High Sensitivity C-reactive protein levels in healthy control and Coronary Heart Disease subjects

Group studied	CHD Subjects (n = 150)	Control Subjects (n = 50)	t	P
Hs-CRP (mg/L) (min-max)	1.7±0.75 (1.01-4.01)	0.93±0.35 (0.12-1.70)	6.89	<.001

(***Abbrv***: S: Significant; Hs-CRP: High Sensitivity C-reactive protein)

According to Ridker *et al.*, (2003) Hs-CRP has emerged as a strong independent risk factor for future cardiovascular events that adds prognostic information at all levels of LDL cholesterol, at all levels of the Framingham Risk Score (FRS), and at all levels of the metabolic syndrome. Pearson *et al.*, (2003) suggested that levels of Hs-CRP of <1, 1 to<3, and ≤3 mg/L be used to represent low, moderate, and high vascular risk. C-reactive protein is a risk factor for coronary heart disease. Recent work further indicates that CRP can be produced within the vascular smooth muscle of diseased coronary arteries and that this production may directly lead to the expression of several mediators of the atherothrombotic process (Jabs *et al.*, 2003; Calabro *et al.*, 2003). Physicians' health study and the women's health study showed that measurement of CRP increased the predictive value of lipid variables in determining the risk of first myocardial infarction (Ridker *et al.*, 2003).

Changes in the concentration of plasma lipids frequently observed in CHD patients certainly contribute to the development of vascular disease. Cholesterol has been singled out as the primary factor in the development of atherosclerosis. HDL is regarded as one of the most important protective factors against arteriosclerosis. HDL's protective function has been attributed to its active participation in the reverse transport of cholesterol. Numerous cohort studies and clinical trials have confirmed the association between a low HDL and an increased risk of coronary heart disease. The concentration of LDL correlates positively whereas HDL correlates inversely to the development of coronary heart disease. We observed increased concentration of total cholesterol, triglycerides, LDL cholesterol and VLDL cholesterol but decreased concentration of HDL cholesterol in CHD patients as compared to the control subjects. Our results are in accordance with previous reports (Tomas *et al.*, 2004). The concentration of LDL correlates positively whereas HDL correlates inversely to the development of coronary heart disease (Ambrose and Barua 2004). Our work has revealed a significant increase in Hs-CRP and lipid profile in the circulation of patients with CHD. A significant decrease in the levels of HDL was observed in CHD patients. The concentration of Hs-CRP (inflammatory, marker) is increased in coronary heart disease subjects. Therefore we suggest use of these biomarkers as a diagnostic tool for CHD patients.

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