Association of high sensitivity C – reactive protein (hs-CRP) with unstable angina: An alarm for vulnerable plaques in CAD patients

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Abstract

Introduction: Inflammation plays a crucial role in the development and progression of Coronary Artery Disease. HsCRP has been identified as one of the promising inflammatory marker associated with CAD. It is synthesized at various sites but its secretion by macrophages residing in vulnerable plaque has been shown to be associated with plaque rupture. Unstable angina is linked with vulnerable plaques.

Aim: The study aimed to evaluate the circulatory levels of hsCRP in CAD patients and its association with severity of the disease.

Materials and Methods: In the present study 103 CAD patients, diagnosed for coronary artery disease (by Coronary Angiography and by ECG findings) were included with 96 controls. Further CAD patients were sub classified according to number of vessels involved (SVD, DVD and TVD) and type of angina (Stable and Unstable).

Results: In the present study anthropometric parameters and lipid profile did not show any significant statistical variation in CAD and control groups except HDL. Low levels of HDL have been reported in CAD patients as compared to controls [39.56(±14.44)/ 44.694(±12.44)]. High hsCRP levels have been observed in CAD patients than controls group [5.07(±3.25) /1.9 (± 1.45)]. In CAD group, CAD patients with unstable angina reported the highest concentration of hsCRP than CAD patients with stable angina independent of number of diseased vessels [7.86 (±2361)/ 3.43 (± 2.50)].

Conclusion: The present study observed highest levels of hsCRP in CAD patients with unstable angina and considered them as the most risky group amongst the CAD patients.

Keywords: Inflammation, Atherosclerosis, hsCRP, CAD, Unstable angina.

Introduction

Indians are suffering the brunt of Coronary Artery Disease (CAD) by adapting the western lifestyle, food habits and stressful job profiles.[1] Most of the CAD patients do not show abnormal lipid profiles.[2] The inflammatory milieu in Indians is on high as compared to the western population.[3,4] Subclinical inflammation plays an important role in the development and progression of atherosclerosis.[5] A variety of bio molecules has been studied to understand their role in setting and progression of the inflammatory cascade in co- morbidities like obesity, type 2 diabetes and cardiovascular diseases (CAD).[6,7] Some genetic studies observed the association of CAD with single nucleotide polymorphisms (SNPs) in Apo E gene and lipoprotein lipase gene DN 9 mutations.[8,9] Various research studies were carried out on CAD patients in Indian population for the assessment of inflammatory status. The association of inflammatory markers like IL-6, TNF alpha,[10] homocysteine,[11] ferritin,[12] Lp (a)[13] and hsCRP[14] with CAD has been shown in Indians. hsCRP has been identified as one of the promising one.[15,16] hsCRP can be used as a maker of prediction of future Coronary events. In 2010, Rao et al reported that, even after adjustment for traditional CAD risk factors, Indians have threefold higher risk of re-infarction. It have been suggested that hsCRP may have additional utility for effective risk stratification in this high risk ethnic population.[16]

The human CRP molecule is composed of five identical non-glycosylated polypeptide chains, containing 206 amino acid residues per chain. It is synthesized mainly in liver while various other sources have been identified including atherosclerotic plaque.[17] CRP and hsCRP are the two names given to the same protein. CRP is measured in a broader range as it is a non specific marker of inflammation and increases in many inflammatory conditions (Normal Range- 1-1000 ml). However hsCRP is measured in a very limited range i.e. high sensitivity (0.1 to >10 mg/L) and considered to be heart/cardiac specific.[18] C-reactive protein (CRP) is a strong predictor of cardiovascular risk. It is a marker of the underlying pro-inflammatory process of atherosclerosis.

The study aimed to evaluate the circulatory levels of hsCRP in CAD patients and its association with severity of the disease.

Materials and Methods

In the present study 103 CAD patients and 96 controls were enrolled. The present study was carried out at Dr. D.Y. Patil Medical College, Nerul, Navi

Mumbai. Patients were recruited from outpatient department (OPD) & Indoor patient department (IPD). The written informed consent was obtained from all the study subjects. The study has been approved by the Institutional Ethics Committee.

Inclusion Criteria: Coronary Artery disease proved by history, clinical examination, blood investigations, electrocardiogram, echocardiography & coronary angiography. Healthy controls > 20yrs of age are included. Age and sex matched healthy individuals without clinical evidence of coronary artery disease and with normal ECG constituted the control group.

Exclusion Criteria: Pregnant women, patients < 20 years of age, with congenital heart disease, acute or chronic infection, chronic liver and kidney disease. Fasting venous blood samples were collected from CAD patients and controls. Routine biochemical tests were performed on autoanalyser. HsCRP levels were estimated by immunoturbidometric method.

Blood Sampling and Methodology: Fasting venous blood samples were collected and circulatory levels of hsCRP were measured by using immunoturbidometric method. Anthropometric parameters like height, weight, waist circumference, waist hip ratio and BMI were recorded for each subject. Routine biochemical parameters like fasting blood sugar, lipid profile etc. were evaluated in clinical laboratory of D.Y Patil hospital and research center, Nerul, Navi Mumbai.

Statistical analysis was performed using the SPSS software (version 16). Demographic and biochemical data were expressed as mean ± S.D. Student't' test used to test the significance between cases and controls. The '*P*' value < 0.05 considered to be significant while < 0.01 is highly significant.

Results

The following table shows the Demographical Characteristics of study subjects. The present study results showed a male dominance pattern of the disease. Family history has been again showed its involvement in the prevalence of CAD.

Table 1: Demographical characteristics of study subjects

Variables	Controls (n=96)	CAD (n=103)	*P* value
Age	44.60(± 13.27)	55.61(± 10.51)	NS
Family History(Yes/No)	23/73	67/36	--
BMI	24.50 (±4.08)	24.80 (±3.62)	NS
WC	95.6 (±13.2)	96.33 (± 9.6)	NS
WHR	0.97 (±0.60)	0.98 (±0.66)	NS

[BMI = Body Mass Index, WC-Waist Circumference, WHR- Waist Hip Ratio]

Anthropometric parameters did not show any significant difference between CAD patients and normal controls [BMI (24.50± 4.08)/ (24.80± 3.62)], WC [95.6 (±13.2)/ 96.33 (± 9.6)] WHR [0.97 (±0.60)/ 0.98 (±0.66)]. The current study results suggests that only obesity is non important than adiposopathy (abnormal adipose tissue).

Table 2: Biochemical characteristics of study subjects

Variables	Controls (n=96)	CAD (n=103)	*P* value
FBS (mg%)	94.42 (±20.25)	130.80 (±58.91)	<0.001
HbA1c (mg%)	5.66 (±1.25)	7.19 (±1.85)	<0.001
Insulin (µIu/L)	9.93 (±6.20)	12.36 (±9.0)	0.004
TG (mg%)	122.44 (±48.1)	144.18 (±70.58)	NS
TC (mg%)	175.91 (±38.05)	179.37 (±52.56)	NS
LDL (mg%)	107.19 (±34.92)	111.94 (±43.05)	NS
HDL (mg%)	44.694 (±12.44)	39.56 (±14.44)	0.001
hsCRP (mg/L)	1.9 (± 1.45)	5.0 7(±3.25)	<0.001

[TG = Triglycerides, TC = Total Cholesterol, LDL = Low Density Lipoprotein, HDL = High Density Lipoprotein, hsCRP=High Sensitivity CRP].

Statically non significant results were observed for total cholesterol [175.91(±38.05)/179.37(±52.56)] and triglycerides [122.44 (±48.1)/ 144.18(±70.58)] levels in the two groups (controls/CAD). However, fasting blood glucose [94.42 (±20.25)/ 130.80(±58.91)] and insulin [9.93 (±6.20)/ 12.36 (±9.0)] and glycosylated hemoglobin (HbA1c) [5.66 (±1.25)/ 7.19 (±1.85)] were significantly high in CAD group compared to controls. Lower levels of HDL [39.56(±14.44)/ 44.694(±12.44)] has been observed in CAD patient than control. No significant difference in other parameters of lipid profile has been noted between CAD and controls. The mean serum hsCRP concentration of the CAD group [5.07(±3.25)] was significantly higher than controls [1.9 (± 1.45)].

Further, the CAD patients were subdivided according to the number of diseased vessels as, Single vessel disease (SVD), Double vessel disease (DVD) and Triple vessel disease (TVD). Higher levels of hsCRP were observed in DVD and TVD (>5 mg/L) than that of SVD (>3 mg/L). The results indicated that circulatory levels of hsCRP increases with the incidence of stenosis in more than one vessel.

Table 3: Comparison of hsCRP levels in CAD patients based on number of diseased vessels

Number of vessels involved	CAD Cases (103)	Serum hsCRP Levels mg/L
Single vessel disease (SVD)	12	3.77 (± 3.80)
Double Vessel Disease (DVD)	37	5.13 (± 3.88)
Triple vessel disease (TVD)	54	5.31 (± 2.83)

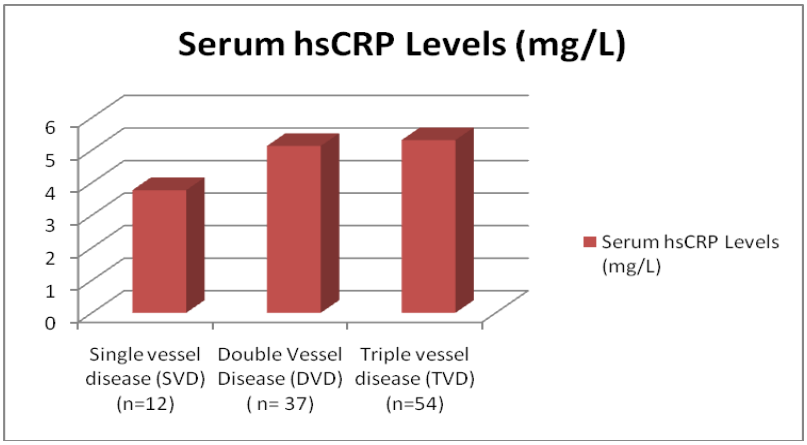

Fig. 1: Comparison of Serum hsCRP in CAD patients according to the number of diseased vessels

It has been observed that, CAD patients with TVD and DVD show higher concentration of hsCRP than that of the CAD patients with SVD. Multiple numbers of diseased vessels show that the endothelial damage had occurred at more than one sites and it is directly correlated with the circulatory levels of hsCRP, a marker of inflammation. The present study did not find any significant correlation between circulatory levels of hsCRP and other biochemical variables.

Table 4: Comparison of hsCRP levels in CAD patients based on type of angina

Type of angina in CAD patients	CAD Cases (103)	Serum hsCRP Levels mg/L
Stable	65	3.43 (± 2.50)
Unstable	38	7.86 (±2361)

HsCRP levels were high in unstable CAD patients than those of stable CAD. It has also been observed that in unstable angina hsCRP levels were high as compared to DVD or TVD patients. It indicates that hsCRP is associated with degree of inflammation.

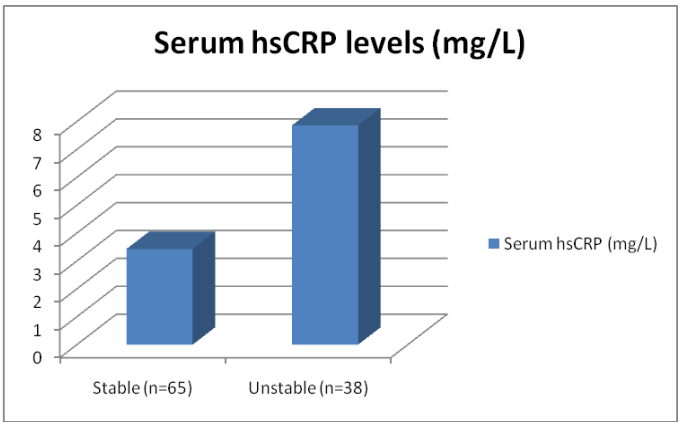

Fig. 2: Comparison of hsCRP levels in CAD patients based on type of angina

Table 5: Distribution of SVD, DVD and TVD patients between Stable and Unstable groups

CAD (103)	Stable (65)	Unstable (38)
SVD (n=12)	07 (58%)	05 (42%)
DVD (n=37)	22 (61%)	14 (39%)
TVD (n=54)	36 (65%)	19 (35%)

The numbers of stable CAD patients were high in all three groups compared to unstable CAD patients. It was observed that unstable CAD patients reported more than double the value of hsCRP than those of stable CAD patients. As compared to normal controls, unstable CAD patients showed four times higher hsCRP levels.

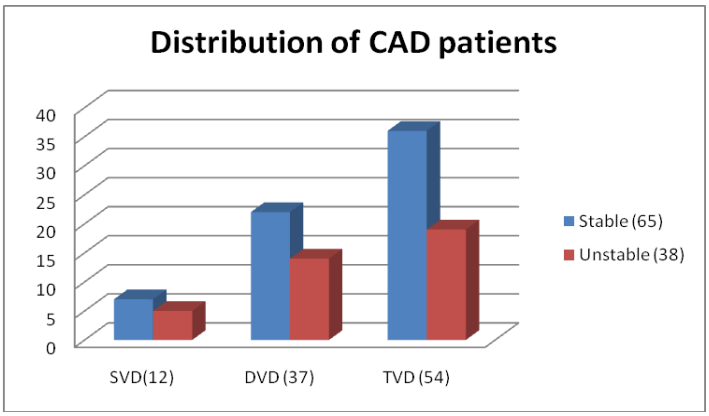

Fig 3: Distribution of SVD, DVD and TVD patients between stable and unstable groups

The circulatory levels of hsCRP were high in unstable CAD patients (being less in number) than that of stable CAD patients. Higher hsCRP levels were more prominent in unstable CAD than DVD or TVD patients [7.86 (±2361)/ 5.13 (± 3.88), 5.31 (± 2.83)].

Discussion

Our study observed high levels of hsCRP in CAD patients than controls. No significant difference was observed in lipid profile and anthropometric measurements of controls and CAD patients except HDL. Low levels of HDL were noted in CAD patients than normal controls [39.56(±14.44)/ 44.694(±12.44)]. Further it has been observed that hsCRP concentration was high in CAD patients with DVD and TVD as compared to SVD patients. However, a drastic increase in hsCRP level has been reported in CAD patients with unstable angina than those with stable angina. Our study supports the hypothesis that hsCRP levels are associated with extent of CAD. Hence hsCRP can be considered as an important marker of inflammatory status in CAD patients especially patients unstable angina.[19,20]

Unstable angina is linked with vulnerable plaques and hsCRP plays a crucial role in plaque rupture. According to some studies hsCRP is associated with endothelial damage, which actually initiates the process of atherogenesis.[21] In 2001 Plutzky et al reported that, hsCRP reflects the intensity of inflammation in plaque and its vurnebility.[22] In unstable angina increased levels of hsCRP connects it with vulnerable plaques. The macrophages residing in plaque keep the inflammatory environment high by secretion of various cytokines and chemokines like IL-6, TNF alpha etc. IL-6 has been identified as one of the inflammatory cytokine, produced by the macrophages residing in plaque and stimulates hsCRP synthesis.[23] In coronary plaque high amount of hsCRP have been found and it is believed that the binding of hs-CRP to lipoprotein could activate the complementary system via the classical pathway, which might result in number of terminal attack complexes, leading to intimal injury and even plaque rupture.

Some studies have shown a significant increase in hsCRP in cases of ACS.[24,25] Wang studied IL-6 and hsCRP in ACS and concluded that both play an important role in plaque vulnerability as well can be used as a marker for plaque progression and for prognosis of the disease.[25] According to the Physician's Health Study, patients with higher basal hsCRP concentration have three times higher MI risk.[26] In 2014, a review paper by Salazar et al reported that circulatory hsCRP 1–3 mg/L have 50% higher risk of CVD, compared to the CAD patients with hsCRP < 1 mg/L, while hsCRP > 3 mg/L is associated with a double CVD risk.[27] In 2012, Adult treatment panel (ATP) III guidelines recommended to consider the CRP levels before prescribing the medications for hyperlipidemia treatment between less and more aggressive for the purpose of evaluating the treatment effectiveness.[28] In case of primary CAD prevention, hsCRP can be considered as a reliable, rapid and cheap method to stratify CAD risk groups and also for the identification of degree and intensity of inflammation.

HsCRP can also be used to prefigure the complications and to evaluate the treatment efficiency.

Conclusion

The present study reported high hsCRP levels in CAD patients than the normal controls. Amongst the CAD patients Double and Triple vessel diseased (DVD and TVD) CAD subjects reported comparatively higher levels than those of Single vessel disease (SVD) CAD patients. Significantly highest levels of hsCRP were reported in CAD patients with unstable angina than those with stable angina, independent of number of vessels involved.

Limitations of the Study: The present study included the subjects from a specific region with a limited sample size. A larger sample size with cross sectional studies is required to confirm the predictive value of hsCRP and intensity of inflammation.

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