CHAPTER 3

High sensitivity C-reactive protein and CAD

3.1. Introduction

In the recent past, there has been a marked shift in the identification of potential clinical markers projected to associate inflammation and atherosclerosis (Szmitko, et al., 2003). Quantifying inflammatory markers in serum may offer clinicians with added information regarding a patient’s risk of CAD. Of these markers, CRP, tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) have been most extensively studied. Even though effortless estimations of white blood cell count and its link with CAD have been reported (Brown, Giles and Croft, 2001), the most promising is CRP, a presumed prognostic and predictive risk factor and marker of CAD events (Pearson, et al., 2003). CRP estimations offer a whole lot of added advantages. It is highly stable with a long plasma half life (19 hours); is measurable at any time irrespective to biological clock or circadian rhythm; can be estimated in fresh or frozen plasma/serum; has least food interaction and assays currently available for its estimation have high sensitivity, specificity and reproducibility.

CRP a member of the pentraxin family of proteins, is an acute-phase reactant, (Figure 14) produced by the liver and by the smooth muscle cells of coronary arteries (Calabro, Willerson and Yeh, 2003) in response to inflammatory stimuli; there is evidence for local expression of CRP in macrophages of the lung and the brain (Yasojima, Schwab, McGeer and McGeer, 2001). It increases 1000-fold
under the influence of cytokines including IL-6 and TNF-α, in response to infection, ischemia, trauma, burns, and inflammatory conditions (Westhuyzen and Healy, 2000; Ridker, et al., 2008). Assays initially developed for estimation of CRP in these conditions, had a detection limit of 3-5 mg/l, which is higher than the level seen in most healthy individuals. Subsequently high sensitivity measurements of CRP for CAD risk is based on the discrimination of levels below 3 mg/L. For determination of CAD risk, the ensuing values have been defined: low risk (<1 mg/L), average risk (1-3 mg/L) and high risk (>3 mg/L) (Pearson, et al., 2003).

Figure 14: (a) C-reactive protein (CRP) synthesis from liver, (b) slightly elevated C-reactive protein in the pancreas (Calabro et al. 2003)

CRP is now understood to be a mediator as well as a marker of atherothrombotic disease. The mechanisms responsible for the association between CRP and cardiovascular disease are not clear. Some studies have suggested that CRP may be only a marker of inflammation and thrombotic risk, without any specific role in the degree of atherosclerosis (Folsom, et al., 2001; Zwaka, Hombach and Torzewski, 2001). While the following observations suggests that there may be a direct effect: i) CRP has been established in atherosclerotic lesions
ii) CRP binds to LDL-C, and mediate its uptake by macrophages without the need for modification (Kushner, 1982) iii) Administration of CRP promote inflammation in humans (Bischoendahl, et al., 2005; Schwedler, et al., 2005).

Ligand-bound or aggregated CRP binds with complement C1q and in doing so, activates the classical complement pathway (Du Clos, 2000). In atherosclerotic plaques, it has been found associated with complement proteins and within foam cells (Torzewski, et al., 2000; Tracy, 2000). Elevated CRP levels are associated with increased cardiovascular risk (Ridker, et al., 1997, 2002, 2001b; Ridker, Hennekens, Buring and Rifai, 2000; Ridker, Stampfer and Rifai, 2001c; Ridker, 2001a). The baseline plasma concentration of CRP as measured by high sensitivity assays (hs-CRP) can predict the risk of future myocardial infarction and stroke (Roberts, et al., 2001; Ridker et, al., 1997).

3.2. Background and Objectives of the Study

Premature CAD and diabetes occurring at a younger age are reported to be frequent in Indians and migrant Asian Indians (Mohan, et al., 2001 and Mohan, Shanthirani and Deepa, 2003; Wild, et al., 2004). The higher rates of CAD among Indians are also not explained by traditional risk factors (Deepa, Arvind and Mohan, 2002). One half of all MIs and strokes occur in persons with below-average cholesterol levels, which underscores the need to go beyond lipids in identifying high-risk candidates for primary prevention. It is now recognized that inflammation, an indispensable component of the atherosclerotic process can predict disease. There is mounting evidence to propose that insulin resistance (IR),
a forerunner for both CAD and diabetes, is coupled with chronic low-grade inflammation (Festa, et al., 2000; Pradhan, et al., 2001). A recent prospective study done on 2459 cases and 3969 controls revealed CRP as a moderate predictor of CAD (Danesh, et al., 2004). It is possible that there could be ethnic differences in the association of hs-CRP with CAD (Chambers, et al., 2001; Chandalia, et al., 2003, Chatha, Andason and Gama, 2002). There is an urgent need for data on hs-CRP in non-European populations, because hs-CRP has been recently suggested to be an adjunct to major cardiovascular risk factors by an expert panel of the American Heart Association and the Centers for Disease Control and Prevention (Pearson, et al., 2003). In this study, we explored the association of serum concentration of hs-CRP with the extent of CAD as assessed by coronary angiography and diabetes and in an attempt to understand whether hs-CRP estimation would be complementary or redundant when combined with clinical risk prediction with other risk factors in a South Indian population.

3.3. Review of Literature

3.3.1. Is C-reactive protein a Bio-marker or Player?

In a series of elegant experiments from Verma’s group, it was proposed that CRP is not only a marker, but also a player in CAD. CRP may directly promote atherosclerosis and endothelial inflammation by attenuating the release of NO, a key molecule in the endothelium that plays a central role in the preservation of vascular tone. NO besides being a potent vasodilator, also mediates many defending functions in the endothelium like hindering the expression of pro-inflammatory cytokines, chemokines and leukocyte adhesion molecules,
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thereby restricting vascular recruitment of leucocytes and platelets (Verma, et al., 2002b). NO also inhibits vascular smooth muscle cell proliferation, an early indication of atherosclerosis. Therefore CRP may have a significant deleterious role in the atherosclerotic process, i.e. by inhibiting NO formation. However, the involvement of CRP as a functional player in the endothelium has recently been questioned, as it has been suggested that the pro-inflammatory effects by CRP might be due to a contaminating artifact in the commercial CRP preparation (Pepys, et al., 2005). Recently, the same authors have demonstrated that injection of pure human CRP into rats augment myocardial infarct size through a complement mechanism; an effect that was dulled after administration of a specific CRP inhibitor, which could be harnessed in the management of CAD (Pepys, et al., 2006). Castoldi, et al., (2007) have established that, in patients with Type 2 diabetes classified into groups based on serum levels of hs-CRP namely with low (<1.0 mg/l), medium (1.0–3.0 mg/l) and high (>3.0 mg/l), there is a noteworthy correlation between hs-CRP and LPS-stimulated release of IL-1β and IL-6 in whole blood. Fascinatingly, CRP levels might be independently correlated to the extent of insulin resistance. In a post-hoc analysis of the predictive value of CRP for the risk of developing diabetes in the WOSCOPS (West of Scotland Coronary Prevention Study) cohort of middle-aged men, a dose-dependent association, independent of conventional risk factors, was undeniably found (Freeman, et al., 2002). It has been suggested that Type 2 diabetes may, in part, be precipitated or accelerated by an acute-phase reaction as part of the inherent immune response, in
which cytokines are released from adipose tissue, generating an inflammatory milieu (Pickup, 2004).

Low-grade inflammation might be a chief pathogenic factor in the development of IR and Type 2 diabetes. Visceral obesity leads to IR and endothelial dysfunction mainly through a cascade of proinflammatory agents being released from visceral adipocytes. According to this hypothesis, cytokines set forth toxic effects on endothelial cells and cause amplified capillary permeability. Cytokines created locally in plaques, especially in inadequately controlled diabetes, could implement oxidative stress and endothelial dysfunction that might intensify the atherosclerotic process further. Also, IR leads to hyperglycaemia, which also prop up an inflammatory milieu. Moreover, hypertension and dyslipidaemia, usually observed in overweight patients with Type 2 diabetes, might add to low-grade inflammation. In reality multifactorial therapy in patients with Type 2 diabetes reduces the risk of CAD events (Rosenson and Koenig, 2002; Buono, et al., 2002; Verma, et al., 2002; Pradhan, et al., 2002; Nakajima, et al., 2002; Gaede, et al., 2003), signifying that these factors are interconnected, numerous and composite. However, the major cause for the accumulation and activation of inflammatory cells in the arterial sub-intimal space and the ensuing expression of pro-inflammatory cytokines and other mediators which play an important role in plaque progression and finally contribute to plaque destabilization and rupture still remains largely unclear (Burke, et al., 2002).
3.3.2. hs-CRP as a Risk Predictor

The relationship between a patient’s baseline level of CRP and future vascular risk has been consistent in studies from the United States and Europe, and in most cases has proven independent of age, smoking, hypertension, hypercholesteremia and diabetes, the major "conventional" risk factors evaluated in daily practice. These profound effects were there among women as well as men, amongst the elderly as well as those in middle age, between smokers and non-smokers, and among those with and without diabetes. CRP levels have long-term predictive value. In an earlier study, event-free survival data have become available that allow clinicians to interpret CRP levels either in terms of population-based quintiles (Figure 15, left) or in terms of simple clinical cut-points (Figure 15, right) (Ridker, et al., 2002).

![Figure 15: CAD event-free survival among apparently healthy individuals according to baseline CRP levels. Data are shown using population-based quintiles for CRP (left) and using 3 simple clinical cut-points for CRP, <1, 1 to 3, and >3 mg/L (right), (Ridker et al. 2002).](image)

Prospective data also demonstrate that CRP is a stronger predictor of risk than is LDL-C. In the largest study so far, both the area under the receiver operator
characteristic (ROC) curve (0.64 versus 0.60) and the population attributable risk percent (40 versus 19) were considerably greater for CRP than for LDL-C (Ridker, et al., 2002).

**Figure 16:** CRP provides prognostic information at all levels of LDL-C and at all levels of the Framingham Risk Score. Data adapted from reference (Ridker et al. 2002).

CRP levels minimally associate with lipid levels and there is practically no way to predict CRP levels on the basis of total cholesterol, HDL-C or LDL-C. In evaluations including over 25,000 patients, the variance in CRP that can be attributed to LDL-C have consistently been less than 3% to 5% (Albert, Danielson, Rifai and Ridker, 2001; Ridker, et al., 2001b and 2002). Thus, CRP levels do not replace lipid evaluation, but must be considered as an adjunct to lipid evaluation. The additive value of CRP to lipid examination in terms of coronary risk prediction has been demonstrated in several settings (Ridker, et al., 1997, 1998, 2001a, 2001b; Ridker, Hennekens, Buring and Rifai, 2000; Ridker, Stampfer and Rifai, 2001c, and 2002). A simplified clinical approach to this issue based on the Adult Treatment Panel III (ATP III) cut off points for LDL-C of <130, 130 to 160, and >160 mg/dL and on CRP levels of <1, 1 to 3, and >3 mg/L is shown in Figure 16,
is an indication that CRP adds prognostic information at all levels of the Framingham Risk Score.

The primary goal of cardiovascular screening programs should be the identification of high-risk individuals who can be targeted for smoking cessation, diet, exercise, and blood pressure control. It is well-known that compliance with lifestyle recommendations is directly related to the absolute risk perceived by individual patients (Albert, Danielson, Rifai and Ridker, 2001). Thus, because the addition of CRP to lipid evaluation provides an improved prediction tool, consideration of CRP may value add for this reason alone. There is currently no definitive evidence that lowering CRP will necessarily reduce cardiovascular event rates; studies addressing this issue are only now being designed. However, many interventions known to lessen cardiovascular risk have been linked to lower CRP levels. In particular, weight loss, diet, exercise, and smoking cessation all lead to both reduced CRP levels and reduced vascular risk (Albert, Danielson, Rifai and Ridker, 2001; Ridker, et al., 2001b and 2002).

Several pharmacological agents were proven to diminish vascular risk by influencing CRP levels. Of these, the statins are the most significant, showing on an average, median CRP levels decline 15% to 25% as early as 6 weeks after starting therapy. As shown in the large-scale Cholesterol and Recurrent Events (CARE) (Ridker, et al., 1999), and pravastatin inflammation/CRP Evaluation (PRINCE) (Albert, Danielson, Rifai and Ridker, 2001) trials and subsequently confirmed in other settings, there is little evidence that the magnitude of LDL-C
reduction predicts the magnitude of CRP reduction. On the other hand, aggressive LDL-C reduction remains a critical therapeutic goal, and thus serial LDL-C evaluation should remain the primary method to monitor statin compliance. All subjects taking statins attain a notable reduction in LDL-C levels but there seems to be responders and non-responders for statins with respect to CRP reduction. However, whether this latter observation is important in terms of clinical event reduction is currently unrevealed.

3.3.3. Association of hs-CRP in CAD population

When measured with high-sensitivity assays, the population distribution of CRP has generally been consistent across sex and ethnic groups, and values of 0.3, 0.6, 1.5, 3.5, and 6.6 mg/L have been reported as estimates of the 10th, 25th, 50th, 75th, and 90th percentile cut-points for middle-aged Americans (Ridker, et al., 2002). In 4 major cohort studies performed in the United States, the Physicians Health Study, the Women’s Health Study (Albert, Glynn, Buring and Ridker, 2004), the Women’s Health Initiative (Pradhan, et al., 2002; Rossouw, et al., 2002, and the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS), the quintile distributions of CRP for men and for women not taking hormone replacement therapy (HRT) (Ridker, et al., 1997, 1998, 1999, 2002, 2001b; Ridker, Hennekens, Buring and Rifai, 2000; Ridker, 2001a; Ridker, Stampfer and Rifai, 2001c) are remarkably similar, and in practice approximate quintile cut-points of <0.5, 0.5 to 1.0, 1.0 to 2.0, 2.0 to 4.0, and >4.0 mg/L have been suggested for use. An alternative approach, as suggested above, is simply one
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that emphasizes levels <1, 1 to 3, and >3.0 mg/L as low-, moderate-, and high-risk groups.

A study was conducted in 150 subjects selected from the Chennai Urban Rural Epidemiology Study (CURES), a population-based study on a representative population of Chennai, (Mohan, Deepa, Velmurugan and Premalatha, 2005). Group 1 comprised of non-diabetic subjects without CAD (n = 50). Type 2 diabetic subjects without CAD formed Group 2 (n = 50); Group 3 encompassed Type 2 diabetic subjects with CAD (n = 50). CAD was diagnosed based on ECG changes indicative of ST segment depression and/or Q wave changes using appropriate Minnesota codes. Study subjects had no infectious or inflammatory diseases. The plasma levels of hs-CRP were measured using a highly sensitive nephelometric assay. Body fat was calculated using Siri's formula using skin fold measurements. Diabetic subjects with (2.89 mg/l) and without (2.25 mg/l) CAD had significantly higher hs-CRP levels compared with non-diabetic subjects without CAD (0.99 mg/l, P < 0.001). hs-CRP values increased with increases in tertiles of body fat (ANOVA P < 0.001) and HbA1c (ANOVA P < 0.001) (Mohan, Deepa, Velmurugan and Premalatha, 2005). Multiple logistic regression analysis revealed hs-CRP to be strongly associated with CAD (OR: 1.649, P= 0.040) and diabetes (OR: 2.264, P= 0.008) even after adjusting for age and gender. Regression analysis also revealed body fat to be strongly associated with diabetes and CAD even after adjusting for age and gender (P < 0.001). In this case, hs-CRP showed a strong association with CAD and diabetes (Mohan, Deepa, Velmurugan and Premalatha, 2005).
hs-CRP has been shown to be predictive of CAD events but data on hospital based angiographically demonstrated case-control studies among Asians and Indians is comparatively very few (Table 6). Hence the role of hs-CRP in the CAD risk among South Indian population with and without Type 2 DM and with respect to other cardiovascular risk factors was assessed in this angiographically proven case-control study.

**Table 6: Association of hs-CRP to CAD risk in Global and Indian Population**

<table>
<thead>
<tr>
<th>Author</th>
<th>Sample size</th>
<th>Non-diabetic subjects without CAD (mg/l)</th>
<th>Diabetic subjects without CAD (mg/l)</th>
<th>Diabetic and Non-diabetic subjects with CAD (mg/l)</th>
<th>CAD Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Global Population</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sung, et al., (2011)</td>
<td>234</td>
<td>0.176</td>
<td>-</td>
<td>0.912</td>
<td>Mild</td>
</tr>
<tr>
<td>Kim, et al., (2010)</td>
<td>231</td>
<td>0.472</td>
<td>-</td>
<td>2.92</td>
<td>High</td>
</tr>
<tr>
<td>Koc, Karaarslan, Abali and Batur (2010)</td>
<td>124</td>
<td>↓</td>
<td>-</td>
<td>↑</td>
<td>High</td>
</tr>
<tr>
<td>Goei, et al., (2009)</td>
<td>592</td>
<td>↓</td>
<td>-</td>
<td>2.54</td>
<td>High</td>
</tr>
<tr>
<td>Huang, et al., (2009)</td>
<td>322</td>
<td>↓</td>
<td>-</td>
<td>4.00</td>
<td>Very high</td>
</tr>
<tr>
<td>Lee, et al., (2009)</td>
<td>418</td>
<td>0.30</td>
<td>-</td>
<td>12.6</td>
<td>Very high</td>
</tr>
</tbody>
</table>
3.4. Materials and Methods

3.4.1. Estimation of Serum hs-CRP

Serum concentrations of hs-CRP were estimated using hs-CRP latex DAIICHI kit *(Daiichi Pure Chemicals Co. Ltd., Tokyo, Japan)* by immunoturbidimetric method using semi auto analyser *(Star 21 plus, Rapid Diagnostics, USA)*.

**Kit Contents:**

Buffer solution: Tris (hydroxymethyl) aminomethane (20 mmol/L, pH 8.5).

Latex reagent: Antihuman CRP mouse monoclonal antibody coated latex (2.25 mg/mL).

**Method:**

CRP reacts with antihuman CRP mouse monoclonal antibody coated latex and agglutination occurs. CRP concentration is determined by measurement of the change in absorbance that results from the agglutination reaction as follows:
CRP + Antihuman CRP mouse monoclonal antibody coated latex → Immune complexes.

**Preparation:**

Reagent 1: Buffer solution is a ready-to-use reagent.

Reagent 2: Latex reagent is a ready-to-use reagent.

**Procedure:**

Sample, Reagent (1) \(37^\circ C\) → Reagent (2) \(37^\circ C\) → Measurement (Abs I*)

- Sample + Reagent (1) 37°C → Reagent (2) 37°C → Measurement (Abs I*)
- 3 μL 150 μL 5 min 50 μL 30 sec

37°C → Measurement (Abs II*) → Calculation of CRP concentration

4 min 30 sec

*Absorbance I, II: Difference in absorbance at 800 nm and 570 nm

Standard: CRP calibrator (SS-type), Reagent blank: Saline.

**Sensitivity:**

- Reagent blank: less than 0.015 Abs.
- Sensitivity: 0.02 – 0.12 Abs. per 1 mg/dL CRP

**Specificity:**

90-110% of expected assay values.

**Precision:**

Coefficient of variation is less than 5%
3.4.2. Statistical Analysis

Descriptive statistics were used to summarize the clinical findings, risk factors, and coronary angiographic findings of patients. Student’s t test was used to get the statistical significance. Pearson’s correlation co-efficient was used to find the association between hs-CRP and other cardio vascular risk factors. The association between individual risk factor and outcome was estimated using univariate logistic regression. The multivariate logistic regression analysis was used to estimate the effect of hs-CRP on CAD, controlling the other confounders. As the distribution of CRP was highly skewed, logarithmic transformation of CRP was used for statistical analysis. A P value of <0.05 was taken as significant.

3.5. Results

The mean serum levels hs-CRP in CAD subjects was significantly higher (Mean±SEM=0.55±0.012 mg/dl) than controls (Mean±SEM=0.450±0.012mg/dl) (P<0.001) (Figure 17). CAD subjects with (Mean ± SEM=0.56±0.015mg/dl) and without (Mean ± SEM=0.53 ±0.020mg/dl) diabetes had significantly higher hs-CRP levels (P<0.05) when compared with diabetic subjects without CAD (Mean ± SEM=0.46 ±0.017 mg/dl) and non-diabetic subjects without CAD (Mean ± SEM=0.43 ± 0.016 mg/dl) (Figure 18).

In our study, a significantly mounting trend in hs-CRP was observed with the increase in severity of CAD: NCAD (No CAD) (Mean ± SEM=0.44 ± 0.012 mg/dl) < SVD (Mean ± SEM=0.53 ± 0.013 mg/dl) < TVD (Mean ± SEM=0.56 ± 0.021 mg/dl) < DVD (Mean ± SEM=0.57 ± 0.024 mg/dl) (P<0.05) (Figure 19).
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Figure 17: hs-CRP levels in relation to CAD

Figure 18: hs-CRP levels in relation to CAD and DM

Figure 19: hs-CRP levels in relation to severity of CAD
Table 7: Pearson correlation analysis of hs-CRP with cardiovascular risk factors

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>hs-CRP (mg/dl)</th>
<th>r value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.123</td>
<td>0.052</td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>0.003</td>
<td>0.965</td>
<td></td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>0.046</td>
<td>0.470</td>
<td></td>
</tr>
<tr>
<td>Waist Hip Ratio</td>
<td>-0.002</td>
<td>0.974</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>0.132</td>
<td>0.037*</td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>0.169</td>
<td>0.007**</td>
<td></td>
</tr>
<tr>
<td>HOMA IR</td>
<td>0.204</td>
<td>&lt; 0.001**</td>
<td></td>
</tr>
<tr>
<td>Fasting Blood Sugar (mg/dl)</td>
<td>0.216</td>
<td>&lt; 0.001**</td>
<td></td>
</tr>
<tr>
<td>Glycated hemoglobin (%)</td>
<td>0.301</td>
<td>&lt; 0.001**</td>
<td></td>
</tr>
<tr>
<td>Serum cholesterol (mg/dl)</td>
<td>0.044</td>
<td>0.489</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>0.105</td>
<td>0.099</td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>0.005</td>
<td>0.936</td>
<td></td>
</tr>
<tr>
<td>Non HDL cholesterol (mg/dl)</td>
<td>0.075</td>
<td>0.265</td>
<td></td>
</tr>
<tr>
<td>TG : HDL</td>
<td>0.120</td>
<td>0.075</td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol : HDL</td>
<td>0.002</td>
<td>0.975</td>
<td></td>
</tr>
<tr>
<td>Serum triglycerides</td>
<td>0.103</td>
<td>0.103</td>
<td></td>
</tr>
<tr>
<td>Cardiac Markers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Troponin t (ng/dl)</td>
<td>0.157</td>
<td>0.013*</td>
<td></td>
</tr>
<tr>
<td>CPK (u/l)</td>
<td>0.263</td>
<td>&lt; 0.001**</td>
<td></td>
</tr>
<tr>
<td>CPK-MB (u/l)</td>
<td>0.224</td>
<td>&lt; 0.001**</td>
<td></td>
</tr>
<tr>
<td>LDH (u/l)</td>
<td>0.221</td>
<td>&lt; 0.001**</td>
<td></td>
</tr>
<tr>
<td>Stenosis score</td>
<td>0.245</td>
<td>&lt; 0.001**</td>
<td></td>
</tr>
</tbody>
</table>

**Correlation is significant at 0.01 level  
*Correlation is significant at 0.05 level

Table 7 summarises the association of hs-CRP with various cardiovascular risk factors in the study population. There was a significant relationship between hs-CRP levels, systolic blood pressure (r=0.132; P<0.05), diastolic blood pressure (r=0.169; P<0.01), HOMA IR levels (r=0.204; P<0.001), fasting blood sugar levels (r=0.216; P<0.001), glycated hemoglobin (r=0.301; P<0.001), and a minimal correlation between hs-CRP and lipid levels, i.e. Serum cholesterol (r=0.044; P=NS), HDL-C (r=0.105; P=NS), LDL-C (r=0.005; P=NS), TG (r=0.120; P=NS), TC (r=0.002; P=NS). Serum triglycerides (r=0.103; P=NS). Serum hs-CRP levels...
significantly correlated with cardiac markers namely Trop t (r=0.157; P<0.05), CPK (r=0.263; P<0.001), CPK-MB (r=0.224; P<0.001), LDH (r=0.221; P<0.001) and stenosis score (r=0.245; P<0.001) (Table-7).

![Figure 20: hs CRP levels in relation to family history of CAD](image)

![Figure 21: hs CRP levels in relation to smoking](image)
There is no question that CAD runs in families. In our analysis (Figure 20), hs-CRP was higher in FH-CAD (Mean ± SEM=0.52 ± 0.013 mg/dl; P<0.05) than non-FH-CAD (Mean ± SEM =0.48 ± 0.012 mg/dl; P<0.05). Hence, the elevated level of hs-CRP in FH-CAD and lower level of hs-CRP in non-FH-CAD indicates the consequence of FH in increasing risk of CAD. Our study showed that (Figure 21), the levels of hs-CRP were lower in non-smokers (Mean ± SEM=0.49 ± 0.009 mg/dl) and ex-smokers (Mean ± SEM=0.49 ± 0.049 mg/dl) than in smokers (Mean ± SEM=0.54 ± 0.029 mg/dl) which emphasize the higher risk of CAD among smokers. These findings revealed that smoking and positive family history are the major risk factors for CAD.

Table 8: Multiple logistic regression analysis using CAD as dependent variable

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Odds Ratio [OR]</th>
<th>95% Confidence Interval [CI]</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Independent variable: hs-CRP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1: hs-CRP – Unadjusted</td>
<td>2.032</td>
<td>1.522 – 2.714</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Model 2: [Model 1 + adjusted for age and gender]</td>
<td>2.419</td>
<td>1.713 – 3.418</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Model 3: [Model 2 + adjusted for insulin resistance]</td>
<td>2.250</td>
<td>1.586 – 3.192</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Model 4: [Model 3 + FBS]</td>
<td>2.191</td>
<td>1.538 – 3.122</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 8 depicts the multiple logistic regression analysis of hs-CRP using CAD as a dependent variable. The odds ratio for the unadjusted and the adjusted models were as follows: Model 1 (hs-CRP – Unadjusted), Model 2 (Model 1 + adjusted for
An investigation into the relationship of insulin and other related biochemical parameters with coronary artery disease in a South Indian population

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Model 3 (Model 2 + adjusted for insulin resistance) and Model 4 (Model 3 + FBS) of regression model were 2.032 (95% CI 1.522 – 2.714, P < 0.001), 2.419 (95% CI 1.713 – 3.418, P < 0.001), 2.250 (95% CI 1.586 – 3.192, P < 0.001) and 2.191 (95% CI 1.538 – 3.122, P < 0.001), respectively to predict CAD. hs-CRP showed a strong association with CAD even after adjusting for age, gender, insulin resistance and fasting blood sugar.

3.6. Discussion

Studies in Western populations have shown low-grade systemic inflammation to be one of the mechanisms by which known risk factors such as obesity, smoking, and hypertension promote the development of diabetes mellitus and CAD (Festa, et al., 2000; Pradhan, et al., 2001; Danesh, et al., 2004). Relatively, few studies of hs-CRP in Asian Indians (Mohan, et al., 2001, 2010a; Mohan, Shantirani and Deepa, 2003; Mohan, Deepa, Velmurugan and Premalatha, 2005; Mohan, Venkataraman and Pradeepa, 2010b; Wild, et al., 2004; Mitra and Panja, 2005), and recent large cross-sectional control studies on global population (Goei, et al., 2009; Lu. et al., 2009; Jin, et al., 2009; Huang, et al., 2009; Biasucci, et al., 2009; Delhaye, et al., 2009; Lee, et al., 2009; Ishii, et al., 2009; Gensini, et al., 2010; Yan, et al., 2010; Jung, et al., 2010; Kotecha, et al., 2010; Schöndorf, et al., 2010; Ristić, et al., 2010; Kim, et al., 2010; Koc, Karaarslan, Abali and Batur, 2010; Sung, et al., 2011) reflects our study objectives and emphasize that hs-CRP is potentially a high-risk factor for developing diabetes and premature CAD. hs-CRP is considered by some to be a ‘golden marker’ for cardiovascular disease (Gomes, 2002). Apart from being an inflammatory marker, hs-CRP has been...
shown to be a risk factor for CAD as it enhances LDL-C aggregation and the production of vascular cell adhesion molecules triggering the atherosclerotic process. It also stimulates matrix metalloproteinase expression, which increases plaque vulnerability (Verma, et al., 2002a; Fu and Borensztajn, 2002; Williams, et al., 2004). Some prospective studies have demonstrated that hs-CRP is a strong independent predictor of future myocardial infarction and stroke and it has also been shown to be related to common carotid artery intima-media thickness, a preclinical atherosclerotic marker (Festa, et al., 2000; Pradhan, et al., 2001; Winbeck, et al., 2002; Danesh, et al., 2004).

The current study also confirms that hs-CRP is significantly associated with CAD and that this effect persisted even when adjusted for age, gender and fasting blood sugar. These findings indicate that low-grade inflammatory activity might influence cytokine production in CAD patients.

These results are similar to that of a nested case–control study by Ridker, Hennekens, Buring and Rifai, (2000), which showed hs-CRP to have a stronger association with cardiovascular events compared with other markers. However, a more recent study demonstrated hs-CRP to be a weaker predictor of CAD than serum lipid parameters (Danesh, et al., 2004).

Population studies have shown strong correlations with proinflammatory biomarker, such as CRP in glucose homoeostasis, obesity, and atherosclerosis. Interestingly, CRP levels might be independently related to the degree of insulin resistance. The acute phase responses associated with Type 2 diabetes thus offer...
insights into the plausible mechanisms by which atherosclerosis is accelerated in Type 2 diabetes, including mediation by acute-phase proteins themselves (Sjoholm and Nystrom, 2005). Some studies have explored the strong relation of hs-CRP with insulin resistance, which precedes CAD and diabetes (Chambers, et al., 2001; Pannacciulli, et al. 2001; Nakanishi, et al., 2003 Xu, H., 2003).

The current study revealed that hs-CRP levels were significantly elevated in diabetic subjects when compared with non-diabetic subjects with and without CAD. This interesting finding supports the concept of ‘common soil’ hypothesis, and that patients with type 2 DM but without presence of CAD exhibit a similar risk for coronary events as patients who have already suffered MI but who are non-diabetic, as several risk factors are relevant in both diseases. Also there was a statistically significant association of hs-CRP levels with insulin resistance as computed by HOMA-IR, which was consistent with the reports of Koenig W. 2002, substantiating further the link between insulin resistance, CAD and inflammation. This finding emphasizes the need for identifying the ‘at risk’ patient at the earliest to prevent the undue complications of CAD and DM. All these findings in the South Indian population corroborated with certain studies done in Western population that hs-CRP predicts diabetes (Pradhan et al. 2001; Haffner, 2003; Hanley et al. 2004; Henareh et al. 2005) and in Indian population but with “no angiographic evidence” (Mitra and Panja, 2005; Mohan, Deepa, Velmurugan and Premalatha 2005, 2010a and Mohan, Venkataraman and Pradeepa, 2010b). These results implicate the need for measurement of serum hs-CRP to improve risk stratification especially among diabetic patients with suspicion of CAD.
Most of the studies done before in patients with chronic stable angina are prospective studies on role of serum CRP in predicting future cardiac prognosis and risk of acute coronary event (Folsom, et al., 2001; Zebrack, et al., 2002; Khera, et al., 2006), but this case control study was designed to see whether CRP, as a marker of inflammation, can predict as well the degree of coronary stenosis in patients with CAD and whether it is an independent predictor after adjustment for other traditional CAD risk factors.

In the present study we detected a significantly increasing trend in serum hs-CRP levels with increasing levels of cardiac markers, increasing severity of CAD as determined by angiography and the severity of stenotic lesions revealed by Gensini Scores. These interesting findings matched with the observations of the study by Arroy, et al., 2003, who reported that in patients who were hospitalized for treatment of acute coronary syndrome with significantly more ischemic episodes had raised CRP levels than patients with lower CRP levels, during their hospital stay. Katritis, et al., (2001) also observed that among patients with suspected CAD undergoing coronary angiography, increased CRP strongly associated with specific high risk features of culprit coronary artery lesions. Also Zairis and colleagues reported that hs-CRP concentration correlated with stenosis complexity in patients with acute coronary syndrome (Zairis, et al., 2002). In patients with acute coronary syndrome increased CRP level is associated with presence of complex angiographic lesions and the need for revascularization (Moukarbel, Arnaout and Alam, 2001).
The current study also suggests that hs-CRP, a marker of systemic inflammation, is a stronger predictor of cardiovascular events than LDL-C. In this study, hs-CRP (Mean ± SEM = 0.55, P<0.001) was superior to LDL-C (P value = 0.550) in predicting the risk of all study end points; this advantage persisted in multivariable analyses in which we adjusted for all traditional cardiovascular risk factors (OR 2.191 (95% CI 1.538 – 3.122, P < 0.001). However, hs-CRP and LDL-C levels were minimally correlated.

Our findings strengthen the hypothesis that acute inflammation is a component of the pathophysiology of coronary syndromes as CAD patients with diabetes had higher hs-CRP levels. Our results suggest that hs-CRP is not only a marker of vascular inflammation but also play an important key role in myocardial necrosis which is leading to CAD.

3.7. Conclusion

In the current study serum hs-CRP levels were significantly higher in patients with CAD and diabetes when compared with non-diabetics with and without CAD. Also the rise in serum hs-CRP levels was significantly associated with cardiac markers, angiographic severity of CAD and the extent of stenosis. These results substantiate the concepts, that inflammation is an important inherent component of atherosclerotic and plaque burden and that inclusion of hs-CRP in the traditional lipid profile will further improve risk prediction. These findings have quite a few implications for future research; including CRP levels may
become a target for therapeutic intervention by blocking its hepatic biosynthesis or CRP mediated complement activation leading to athero-thrombotic events.

In digest, our research on CRP has established hs-CRP’s role as a powerful risk factor for CAD even after adjustment with the traditional risk factors like age, sex and fasting blood sugar. Further studies with even larger sample size and multiple checks of hs-CRP can further shed light on this complex cascade of patho-biological events leading to CAD associated with mounting morbidity and mortality.
References


An investigation into the relationship of insulin and other related biochemical parameters with coronary artery disease in a South Indian population


High sensitivity C-reactive protein and CAD


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High sensitivity C-reactive protein and CAD


