Introduction

Many lines of evidence, ranging from in vitro experiments to pathological analysis and epidemiological studies show that atherosclerosis is an intrinsically inflammatory disease.

Over the last few years, C-reactive protein (CRP) assays have been tested in a series of large-scale prospective clinical studies which demonstrated the importance of this marker in predicting risk of heart attack, stroke and peripheral vascular diseases. It is increasingly recognised that CRP may not merely represent an indication of inflammation but may also, because of its known functional properties, be actively involved in the initiation or perpetuation of local inflammatory reactions (11,26).

It is possible that CRP contributes to pathogenesis of CAD with different effect at every stage of progression. Production of CRP, a nonspecific reaction to various stimuli including infection, tissue damage and smoking may contribute to the initiation and progression of coronary artery disease. Acute phase proteins and CRP are stimulators of tissue factor production from mononuclear cells, an important initiator of coagulation. It also interacts with LDL and damaged membranes to activate the complement system. Results of various studies suggest that CRP levels not only reflect the presence of subclinical atheroma but and unstable angina pectoris. Although, all of the potential triggers of inflammation are not fully known, and cytokines, oxidized lipoproteins, and local (arterial) and distant infections (gingivitis, bronchitis) have been implicated, the circulating CRP, an acute phase reactant produced by the liver in response to interleukin-6 (a cytokine induced by interleukin-1 and tumor necrosis factor-alpha), has the most consistent relationship to the future risk of CAD, under diverse clinical settings (4,25,27).

Summary: Aim: The aim of this study was to evaluate the diagnostic value of serum C-reactive protein (CRP) level measurement in predicting coronary artery disease (CAD) that can be shown angiographically. Methods: CRP levels were determined in the blood of 198 patients (patients group, PG) with angiographically documented coronary artery disease and compared with that of 85 patients (control group, CG) who had a clinical indication for coronary angiography but have no angiographically determined coronary artery stenosis, as well as with that of 41 healthy volunteers as a healthy control group (HG) who did not have any complaint and did not have coronary angiography. CRP levels were measured 24 hours prior to angiography in PG and CG patients, and in the morning after not having eaten for same time. Any coronary artery stenosis or plaque formation was defined as CAD. Severity of the disease was assessed by both the number of diseased vessels (0 to 3) and the degree of stenosis (<50 % mild, 50–70 % moderate and >70 % severe). Results: Receiver Operating Characteristics (ROC) curves of CRP in angiographically documented CAD group showed a diagnostic value of 0.659 in female patients, followed by 0.542 in male patients, in predicting CAD. CRP levels were found to be significantly different between groups, higher in PG (6.2 ± 0.86 mg/L) than those of CG (3.7 ± 0.92 mg/L) and HG (0.854 ± 0.2 mg/L) (p<0.05). CRP levels were not associated with the number of diseased vessels, neither with the degree of the occlusion (p>0.05). Multiple logistic regression analysis after adjustment for the established coronary risk factors showed CRP as an independent discriminating risk factor for CAD. Conclusion: It is concluded that CRP measurement has a value in predicting the presence of angiographically documented CAD. However, CRP levels were not associated with the degree or severity of CAD.

Key words: C-Reactive Protein; CRP; Sensitivity; Specificity; Diagnosis; Coronary Artery Disease; CAD; Angiography
also the likelihood of clinical disease development (3,6,27). In normal healthy individuals, CRP is a trace protein with a range of up to 5 mg/L. 90% of apparently healthy subjects have levels of less than 3 mg/L, and 99% of them have that level less than 10 mg/L. There is no difference between levels in healthy men and healthy women, and no variation with age.

Many questions are still unanswered, including whether CRP is a culprit molecule or simply a marker, and the cutoff points and specificity of the methods are still debated. Drugs and other treatments have little or no effect on CRP production unless they also affect the disease process which is responsible for induction of CRP synthesis. It is thus of great interest that the serum level of CRP has been found to be higher in subjects having no complaint but at greater risk of developing MI, stroke and peripheral vascular disease (4,27).

The aim of this study was to compare CRP concentration in patients with CAD, documented by coronary angiography, with that of patients with normal angiograms but having complaints related to ischemic heart disease, as well as with that of normal healthy individuals, to determine the diagnostic value of CRP levels in the progression of CAD. We also aimed to study the association of this marker with the number of diseased vessels and the degree of stenosis.

Material and Methods

The study was designed to evaluate the serum CRP levels of individuals of three different groups. 198 patients were included in the Patient Group (PG) who had documented CAD proven by coronary angiography. The Control Group (CG) consisted of 85 patients with clinical indications for angiography, but normal coronary angiogram. Fortyone healthy subjects were included in the Healthy Group (HG).

Although the healthy subjects did not have coronary angiograms, they underwent a comprehensive physical examination by a physician, and completed the World Health Organisation standards Rose questionnaire on chest pain, and answered other questions about their past medical history. None of the individuals in the healthy group had angina or a prior history of CAD. All of them had normal electrocardiograms based on the Minnesota Coding Criteria.

All Coronary Angiographies were performed in the same centre. Patients diagnosed with acute coronary syndrome six months prior to the study were excluded.

All patients were monitored for somatic illness throughout the investigation period and excluded if symptoms of infection or systemic illness were present (rheumatic disease, chronic liver disease, renal disorder, cancer, etc.). Additional exclusion criteria included the use of statins and other medications that could affect CRP concentrations.

Severity of the disease was assessed by counting both the number of diseased vessels (0 to 3) and the degree of the stenosis (<50% mild, 50–70% moderate and >70% severe) (13).

Prior medical history, personal characteristics and habits were obtained by the questionnaire.

The presence of obesity, diabetes mellitus, and hypertension were also assessed. Obesity was defined as body mass index (BMI) greater than 27.8 kg/m² as proposed by the National Institute’s of Health consensus statement. Diabetes was defined as fasting blood glucose >126 mg/dL or diagnosis of diabetes needing diet or drug therapy. Hypertension was defined as resting systolic blood pressure >140 mm Hg and/or diastolic blood pressure >90 mm Hg (17). Smoking was defined as being a current smoker and smoking at least 10 cigarettes per day for at least ten years.

Standard Blood Sampling

After an eight-hour fast venous blood samples were collected into vacuum tubes in the morning time on the day before the angiographic procedure on the PG and the CG patients. Blood was centrifuged at 3000 g for 10 minutes at 4°C. After separation, the aliquots were frozen at −70 °C until analysis.

Measurements

Serum CRP concentrations were determined with BNA 100 Nephelometer (Dade Behring, Germany) according to the manufacturer’s instructions. The assay was linear from 0.175 to 230 mg/L, calibrators and controls (R-CL-I) were supplied by the manufacturer. Specifications of intra assay and inter assay coefficients of variation of CRP assay were assessed from quality control data of the laboratory which were 3.4% and 6.2%, respectively. The levels of glucose, urea, creatinine, CK, CK-MB, LDH, cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C) both in PG and CG were analyzed by BM/902 (ROCHE Diagnostic Systems, Germany). Low-Density lipoprotein cholesterol (LDL-C) was calculated using Friedewald formula if triglycerides were <400 mg/dL (9).

Statistical Analysis

Values are expressed as mean ± SD for normally distributed variables, and log mean CRP serum levels at 20th and 80th percentiles are given.

Because the distribution of CRP is rightward skewed, values derived from log-transformed means were used as means for this variable throughout the study; these values virtually coincided with median values. A general linear model was used to consider case control differences after adjustment for other factors and the area under the ROC curves were used for comparison of the diagnostic values of different analyses (including PG and CG groups using angiography as the highest accepted standard). Optimal cut-off levels, sensitivity and specificity of CRP were selected based on ROC curves.
Results

No statistically significant difference was noted in HDL-C levels among the three groups. However, age, diabetes, hypertension, BMI, total cholesterol and triglyceride levels were significantly different in three groups (Table 1). Smokers consisted of 47 % in the PG, 16 % in the CG, and 37 % in HG (Table 1).

CRP levels were different in the studied groups, and these differences remained statistically significant after correction (ANOVA) for other risk factors (Table 2). CRP levels were not related to the number of diseased vessels and degree of occlusion (p>0.05) (Table 3).

CRP levels were significantly higher in the PG (6.2 ± 0.86 mg/L) than in the CG (3.7 ± 0.92 mg/L) and the HG (0.85 ± 0.2 mg/L) (p<0.05) (Figure 1, Table 2). The number of subjects with concentrations of CRP ≥10 mg/L comprised of HG, CG and PG groups were 0.2 %, 9 %, and 11 % in the HG, CG and PG respectively.

Optimal cut-off levels of CRP providing the maximum efficiency were found in female and male patients with CADs of 6.9 mg/L and 6.4 mg/L, respectively (Figure 2). The area under the ROC curve was 0.659 in females and 0.542 in male patients (Figure 3 and 4 respectively). ROC curve based specificity of CRP levels (38 % for male, 49 % for female) was rather lower than the sensitivity of CRP levels (76 % male, 78 % for female) (Table 4).

A significant difference was seen in the gender-related difference of CRP concentrations in the PG (Table 5), and these differences were statistically significant after correction with ANCOVA for age, blood pressure, BMI, smoking, glucose, lipids concentration, number of diseased vessels and degree of occlusion.

CRP did not correlate with other continuous variables such as total cholesterol, (r: 0.095, p: 0.15), triglycerides (r: 0.07, p: 0.21), BMI (r: 0.09, p: 0.17), HDL-c (r: -0.087, p: 0.243), LDL-c (r: 0.07, p: 0.283), glucose (r: 0.06, p: 0.389).

Tab. 1: Baseline patients and normal controls characteristics (mean ± SD).

|                      | Healthy Control Group (HG) n: 41 | Control Group (CG) n: 85 | Patient Group (PG) n: 198 |
|----------------------|----------------------------------|--------------------------|---------------------------|
| Age (year)           | 33.9 ± 1.3*                      | 52.2 ± 9                 | 57.3 ± 6                  |
| Gender (%)           | 20 F/21 M(0.95)                  | 46F/39 M(1.17)           | 47F/151M(0.32)           |
| Current smoker (%)   | 37                               | 16                       | 47                        |
| Cholesterol (mg/L)   | 1736.8 ± 47.0*                   | 2221.8 ± 69.0            | 2151.2 ± 41.0            |
| LDL-C (mg/L)         | 1100 ± 40.7**                    | 1314.7 ± 62.4            | 1313.5 ± 33.8            |
| HDL-C (mg/L)         | 396.6 ± 16.4                     | 424.3 ± 20.5             | 394.1 ± 10.5             |
| Triglycerides (mg/L) | 1200.2 ± 80.5*                   | 2574.6 ± 224.7           | 2287.3 ± 85.9            |
| Cholesterol/HDL-C    | 4.6 ± 0.2**                      | 6.2 ± 0.5                | 5.9 ± 0.1                |
| Diabetes (%)         | 3 (7.3)***                       | 9 (10)                   | 24 (12)                  |
| BMI (kg/m²)          | 24 ± 4.1***                      | 27.1 ± 3.5               | 27.6 ± 3.8               |
| Hypertension (%)     | 10 (24)***                       | 41 (48)                  | 96 (48)                  |
| CRP (≥10 mg/L, n, %) | 1 (0.2)*                         | 8 (9)                    | 22 (11)                  |

*p<0.001 HG versus CG,PG
**p<0.005 HG versus CG,PG
***p<0.01 HG versus CG,PG

Tab. 2: Serum CRP concentrations (mean ± SD)*.

|                      | HG n: 41 | CG n: 85 | PG n: 198 |
|----------------------|----------|----------|-----------|
| **CRP (mg/L)**       | 0.85 ± 0.2| 3.7 ± 0.92| 6.2 ± 0.86|

*(mean ± SD denote values derived from log transformed)*

Tab. 3: Serum CRP and severity of coronary artery diseases.

|                      | n | CRP (mg/L) (mean ± SD)* |
|----------------------|---|-------------------------|
| One vessel           | 38| 7.3 ± 3.7               |
| Two vessel           | 54| 5.2 ± 3.1               |
| Three vessel         | 106| 6.2 ± 1.9              |
| <50 % **             | 26| 5.4 ± 4.9               |
| ≥50 % and <70 % **   | 17| 5.2 ± 3.1              |
| ≥70 % **             | 155| 6.2 ± 1.9             |

*(mean ± SD denote values derived from log transformed) **Degree of occlusion
**Tab. 4:** Optimal cut-off levels and the associated specificity, sensitivity, diagnostic value of CRP levels for the diagnosis angiographically documented CAD.

|            | Cut-off level (mg/L) | Specificity (%) | Sensitivity (%) | Diagnostic Value |
|------------|----------------------|-----------------|-----------------|------------------|
| Male CRP   | 6.4                  | 38              | 76              | 0.542            |
| Female CRP | 6.9                  | 49              | 78              | 0.659            |

**Tab. 5:** Gender related difference of CRP concentrations in the patient group (mean ± SD)*.

|            | Male | Female |
|------------|------|--------|
| CRP (mg/L) | n: 151 | 6.1 ± 1.7 | n: 47 | 7.2 ± 3.6 |

* (mean ± SD denote values derived from long transformed)

**Fig. 1:** Distribution of serum CRP concentrations in PG, CG and HG groups. The central box represents the values from the lower to upper quartile (25–75 percentile). The middle line represents the median.

**Fig. 2:** Distribution of serum CRP concentrations in PG, male and female groups. The central box represents the values from the lower to upper quartile (25–75 percentile). The middle line represents the median.

**Fig. 3:** ROC curve of CRP and optimal cut-off points in male patients.

**Fig. 4:** ROC curve of CRP and optimal cut-off points in female patients.
We also found that, notwithstanding the normal coronary CRP concentration and severity of CAD. These findings increased in patients with angiographically documented (12). In this study, although CRP levels were found to be associated with the degree of stenosis of coronary arteries was positively related to the number of diseased vessels and artery disease. They also reported that CRP concentration CRP in men with angiographically documented CAD. Recent work from Pasceri et.al indicates that CRP itself might be involved in the pathogenic response. In their study they suggested that CRP could induce adhesion molecule expression in endothelial cells via its effect on monocyte chemoattracting protein 1. Regardless of the CRP’s precise mechanism for inflammatory effects in the vasculature, perhaps the most relevant is the prospect of inflammation as a therapeutic target (18).

The peak plasma CRP values are strongly associated with post-infarct morbidity and mortality (17). In a meta-analysis of 7 prospective studies, elevated serum CRP concentration was shown to predict future risk of CAD (10). C-reactive protein levels below the conventional upper normal limit of 10 mg/L have been associated with a 2-4 fold increase in risk of myocardial infarction, ischemic stroke, peripheral artery diseases and CAD (18,21). Several prospective studies have shown that healthy people with higher serum CRP levels, though usually within the normal reference range, are at an increased risk of developing MI, stroke or peripheral vascular disease (1,5,14,22).

Rifai et al. demonstrated higher plasma concentration of CRP in men with angiographically documented CAD (20). Similarly, Haideri et.al found an association of elevated CRP levels with angiographically documented coronary artery disease. They also reported that CRP concentration was positively related to the number of diseased vessels and associated with the degree of stenosis of coronary arteries (12). In this study, although CRP levels were found to be increased in patients with angiographically documented CAD, no correlation could be demonstrated between the CRP concentration and severity of CAD. These findings are in agreement with the results of Rifai and colleagues. We also found that, not with standart the normal coronary angiograms, the CG patients had elevated CRP levels, which has not been reported before (12,20).

It has been reported in previous studies that there was no sex-related difference nor association between the CRP levels and age in healthy individuals (7,15,16). However Onat et al. reported that gender is a significant independent determinant of CRP levels in a healthy population in western Turkey (17). In the NHLBI Family Heart Study, CRP values were associated with age, family type, and gender (8). Our study showed that CRP levels in male individuals were higher than that of females in HG individuals. ROC curves (figure 3,4) showed a low level of sensitivity and specificity in the present study, and showed uselessness to diagnose angiographically significant CAD. The area under the ROC curve was 0.542 in males in comparison to 0.659 in females in PG (p<0.05). To best of our knowledge these data were not reported in the literature so far.

One possible explanation of the increased CRP levels in patients with a clinical indication for cardiac catheterisation but with normal angiograms is the association of CRP levels with ongoing atherosclerosis. The increase in CRP levels in these individuals may reflect the diffuse atherosclerotic process in the vascular system rather than the degree of the localised obstruction.

In one study a cut-off value of 15 mg/L was chosen on the basis of ROC curves in patients with unstable angina and myocardial infarction (7). Recommended reference limits for serum CRP levels appeared to be insufficient for discriminating subjects with and without CAD because of considerable overlaps. In contrast to these findings, we found optimal cut-off levels for ROC curves to be 6.4 mg/L for males, and 6.9 mg/L for females with CAD. However, the potential limitations of this study warrant consideration. Although CRP was an independent discriminator between the PG and the CG patients, there was considerable overlap in CRP concentrations between the two populations. The presence of occult or ongoing coronary artery disease in the CG could not be excluded and may have attenuated the differences among the two groups, whereby the diagnostic value of CRP in CAD may be underestimated.

Conclusions

In conclusion, serum CRP level measurement appears to have an important diagnostic value in predicting the presence of angiographically detectable coronary artery disease. However, the higher levels of serum CRP seem not to be associated with the degree or severity of coronary artery disease.

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