CONCURRENT INCREASED HIGH SENSITIVITY C-REACTIVE PROTEIN AND CHRONIC INFECTIONS ARE ASSOCIATED WITH CORONARY ARTERY DISEASE: A POPULATION-BASED STUDY

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ABSTRACT

BACKGROUND: An elevated serum level of C-reactive protein (CRP) is an independent predictor of coronary artery disease (CAD). Chronic infections have also been implicated in the pathogenesis of CAD. AIMS: To investigate how concomitant chronic infection and CRP related to electrocardiogram-defined CAD in a general population. SETTING AND DESIGN: A population-based cross-sectional study, which was conducted in three Iranian ports in the northern Persian Gulf. MATERIALS AND METHODS: For evaluation of CAD, we used Minnesota coding criteria of a 12-lead resting electrocardiogram in 1,754 subjects, aged 25 years and over, selected by cluster random sampling. Sera were analyzed for IgG antibodies to Chlamydia pneumoniae, Helicobacter pylori (H. pylori) and cytomegalovirus (CMV) using ELISA. Measurement of CRP by a high-sensitivity CRP assay was done. STATISTICAL ANALYSIS: Multiple logistic regression analysis was used. RESULTS: None of the infectious agents (CMV, H. pylori, C. pneumoniae and HSV-1) showed a significant association with electrocardiogram-defined CAD after adjusting for sex and age. Elevated CRP levels did not show significant association with electrocardiogram-defined CAD independent of sex and age. Concurrent elevated CRP levels (>10.0 mg/L) and anti-C. pneumoniae [OR = 1.68 (CI, 1.24–2.59; P=0.04)], H. pylori [OR = 1.98 (CI, 1.26–3.13; P=0.003)], CMV [OR = 1.66 (CI, 1.10–2.49; P=0.01)] or HSV-1 [OR = 1.79 (CI, 1.18–2.72; P=0.006)] IgG antibodies were associated with prevalence of electrocardiogram-defined CAD in the general population, after adjustment for multiple risk factors, including age, sex and the components of the metabolic syndrome. CONCLUSIONS: Beyond traditional cardiovascular risk factors, concomitant chronic infection and elevated CRP are significantly correlated with electrocardiogram-defined CAD.

Key words: Chlamydia pneumoniae, coronary artery disease, C-reactive protein, cytomegalovirus, Helicobacter pylori, herpes simplex virus

C-reactive protein (CRP) is a phylogenetically highly conserved plasma protein; with homologous invertebrates and many invertebrates that participate in the systemic response to inflammation. Its rapid increase in synthesis within hours after tissue injury or infection suggests that it contributes to host defense and that it is part of the innate immune response. It is hypothesized that infectious agents exert their effects by inducing a local or systemic inflammatory response and/or by infection-induced autoimmune response involving molecular mimicry. The association of markers of chronic infection in combination with elevated CRP and cardiovascular diseases has not been adequately evaluated. Such knowledge might provide useful insight into the pathophysiology of, and general risk factors associated with, CAD. In this large population-based study, we investigated electrocardiogram-defined CAD in subjects with elevated CRP and serological evidence of chronic infections CMV, H. pylori, C. pneumoniae or HSV-1 in a random population of the northern Persian Gulf adults.

MATERIALS AND METHODS

Community sampling and baseline examinations

Several retrospective and cross-sectional studies have shown an association between previous infections with C. pneumoniae, herpes simplex virus (HSV), cytomegalovirus (CMV), H. pylori, hepatitis A or respiratory tract infection and the presence of CAD or the risk for acute coronary events, but other studies have not shown such an association. Despite these rapidly growing number of studies about associations between infections and CAD, consensus on the possible atherogenic effects of infectious agents has not been achieved and pathogenic mechanisms remain unclear. The association of markers of chronic infection in combination with elevated CRP and cardiovascular diseases has not been adequately evaluated. Such knowledge might provide useful insight into the pathophysiology of, and general risk factors associated with, CAD. In this large population-based study, we investigated electrocardiogram-defined CAD in subjects with elevated CRP and serological evidence of chronic infections CMV, H. pylori, C. pneumoniae or HSV-1 in a random population of the northern Persian Gulf adults.
an Iranian province with the greatest boarder with the Persian Gulf) were selected. The studied ports of the northern Persian Gulf were Bushehr Port (the center of Bushehr Province, with a population of 150,000 and coronary events of 481.05 and 156.61 per 100,000 for men and women respectively), Genaveh and Deilam.

Examinations were conducted in 2003-04. A resting 12-lead electrocardiogram was performed for all the subjects. A fasting blood sample was taken; all samples were promptly centrifuged, separated; and analyses were carried out at the Persian Gulf Health Research Center on the day of blood collection using a Selectra 2 autoanalyzer (Vital Scientific, Spankeren, The Netherlands).

The metabolic syndrome was diagnosed with the criteria indicated by the NCEP-ATP III. Codes 1.1 and 1.2 were classified as myocardial infarction, and codes 1.3, 4.1-4.4, 5.1-5.3 and 7.1 were classified as ischemia. Prevalence of electrocardiogram-defined CAD was defined as myocardial infarction and ischemia together.

Serology
IgG antibodies against C. pneumoniae were measured by a commercial test kit (DRG Instruments GmbH, Germany). The principle of the kit was based on an indirect solid-phase enzyme immunoassay with horseradish peroxidase as a marker enzyme; the positivity threshold was enzyme immunounits (EIU) >45. Sera were screened for IgG antibodies against herpes simplex virus type 1, cytomegalovirus and H. pylori with an ELISA (RADIM SpA, Italia), and the samples were considered positive with IgG values higher than 30 RU/ml for CMV and H. pylori. Samples with optical density higher than cut-off control were considered reactive for anti-HSV type 1 IgG antibodies.

Measurement of CRP by a high-sensitivity CRP assay, CRP HS ELISA (DRG International, Inc., USA), was done. The minimum detectable concentration of the CRP HS ELISA assay was estimated to be 0.1 mg/L. Additionally, the functional sensitivity was determined to be 0.1 mg/L (as determined with inter-assay %C.V.<20%). Elevated serum CRP was defined as more than 10.0 mg/L.

Statistical analysis
The significance of the difference in the results of any two groups was determined by chi-square analysis using 2 × 2 contingency tables. A two-tailed t-test was used to compare the mean values across groups. P < 0.05 was considered statistically significant.

Odds ratios (ORs) estimating the association of presence of IgG antibodies against infectious agents and/or elevated CRP with electrocardiogram-defined CAD were calculated. We found that log transformation of CRP gave a better fit to a Gaussian distribution. The geometric mean for CRP was defined as the arithmetic mean of the log-transformed data ± 2 SD, raised to the power of 10.

In multiple logistic regression analysis, the combined elevated CRP (>10.0 mg/L) and seropositivity to one of the four infectious agents considered as a single entity, sex, age and the components of the metabolic syndrome as covariates and electrocardiogram-defined CAD also as the dependent variable. Statistical analysis was performed with an IBM computer using the SPSS 9.05 statistical software package (SPSS Inc., Chicago, IL).

RESULTS
A total of 1,754 persons (49.2% males, 50.8% females) of the studied population was evaluated for markers of infectious agents and serum levels of CRP. Of the studied subjects, 36.1% was between 25 and 34 years, 29.9% between 35 and 44 years, 21.9% between 45 and 54 years and 12.7% between 55 and 66 years. Of the studied population, 17.3, 18.2 and 7.0% had history of hypertension, hypercholesterolemia and diabetes respectively. The prevalence of consumption of antihypertensive, hypolipidemic and anti-diabetic drugs was 6.5, 3.5 and 3.8% respectively. A positive history of myocardial infarction (2.3%) and stroke (1.1%) was obtained.

A total of 12.7% of the subjects (9.6% of males and 15.6% of females; P< 0.0001) had electrocardiogram-defined CAD. A total of 52.1% of the subjects (54.6% of males and 49.9% of females; P=0.005) had clinical traits of the metabolic syndrome as defined by ATP III criteria.

An estimated 25.9% of the population was obese, 8.6% had diabetes, 18.3% were smokers, 26.3% had hypertension, 22.1 and 47.7% had high total cholesterol and low HDL-cholesterol levels respectively.

Clinical characteristics, laboratory values and seroprevalence of four infectious agents in persons with and without CAD are presented in Table 1. The subjects with coronary heart disease had a higher prevalence rate for H. pylori than the subjects with no electrocardiogram-defined CAD (67.1% versus 60.8%; P=0.04).

The geometric mean of CRP was 1.94 mg/L (3.80 SD) in the studied population. Quartiles (Q) for the population distribution for CRP were as follows: Q1, 0.04-0.80 mg/L; Q2, 0.81-1.70 mg/L; Q3, 1.71-4.50 mg/L; and Q4, 4.51-338.00 mg/L. CRP levels were higher in women (geometric mean = 2.29 mg/L) than men (geometric mean = 1.62 mg/L). The values of geometric mean of CRP levels in persons with and without electrocardiogram-defined CAD are presented in Table 1. The subjects with coronary heart disease had a higher rate for high CRP levels than the subjects with no electrocardiogram-defined CAD (24.2% versus 12.9%; P< 0.0001).

In multiple logistic regression analysis, elevated CRP levels showed a significant association with electrocardiogram-defined CAD [OR = 1.69, CI (1.24-2.30); P< 0.001] after adjusting for sex and age. The elevated CRP levels also showed a significant association with CAD [OR = 1.65, CI (1.10-2.45); P=0.01] after adjusting for the metabolic syndrome components in addition
to sex and age. However, elevated CRP did not show a significant association with electrocardiogram-defined CAD after adjusting for sex, age, the components of the metabolic syndrome and infectious agents (CMV, *H. pylori* and *C. pneumoniae* or HSV-1) in logistic regression models.

Table 2 shows adjusted odds ratios (95% CI) between concurrent elevated CRP levels and infection burden divided into 1 or 2, 3 and 4 infectious agents was 6.4, 26.5, 43.1 and 24.1% respectively. Concurrent elevated CRP levels and infection burden divided into 1 or 2, 3 and 4 serospositivities were associated with an increasing electrocardiogram-defined CAD of 12.6, 15.0, 27.0% respectively (P for trend 0.009). However, infection burden without concomitant elevated CRP levels was not associated with an increased rate of electrocardiogram-defined CAD (12.4, 12.5 and 9.7% respectively; P for trend >0.05).

**DISCUSSION**

We demonstrated an independent association between electrocardiogram-defined CAD and the inflammatory marker CRP. In contrast, after adjustment for multiple risk factors, including age, sex and the components of the metabolic syndrome in a logistic regression model; however, there was a slight change in the odds ratios [Table 2].

None of the infectious agents (CMV, *H. pylori* and *C. pneumoniae* and HSV-1) showed a significant association with electrocardiogram-defined CAD after adjusting for sex, age and/or the components of the metabolic syndrome in a logistic regression model.

The prevalence of seropositivity to 1, 2, 3 and 4 infectious agents was 6.4, 26.5, 43.1 and 24.1% respectively. Concurrent elevated CRP levels and infection burden divided into 1 or 2, 3 and 4 serospositivities were associated with an increasing electrocardiogram-defined CAD of 12.6, 15.0, 27.0% respectively (P for trend 0.009). However, infection burden without concomitant elevated CRP levels was not associated with an increased rate of electrocardiogram-defined CAD (12.4, 12.5 and 9.7% respectively; P for trend >0.05).

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Romano Carratelli C, Nuzzo I, Cozzolino D, Danesh J, Collins R, Peto R. Chronic infections. Low grade, cytomegalovirus, hepatitis A would be helpful when screening for persons. Corrado E, Rizzo M, Tantillo R, Muratori I, Bonura was combined with elevated but not transiently elevated study (the Helsinki Heart Study), persistently serum IgG antibodies to five pathogens (the serum CRP levels and the prevalence of CRP levels. Zhu and colleagues determined increased to 4.2 when seropositivity to C. pneumoniae seropositivity and CRP had accelerates atherothrombotic progression, inflammatory in patients with angiographically documented coronary artery disease. Viable C. pneumoniae were reported in a substantial portion of carotid artery atherosclerotic plaques and were associated with increased serum CRP. These above-mentioned studies support our results that concurrent CRP elevation and chronic infection is strongly associated with CAD.

We conducted our study in a large random population and used seropositivity as a marker for infections; however, it has the advantage of clinical applicability, but the assessment of infection status based on serology without further clinical or laboratory characterization is subject to diagnostic inaccuracies, especially if seropositivity is common because of the widespread distribution of the incriminated microorganism.

When evaluating the possible contribution of an infectious agent in the development of CAD, the establishment of any serologic criteria for classification of persistent infection requires validation of criteria by comparison with a ‘gold standard’ of persistent infection. In the case of the association of C. pneumoniae and atherosclerotic disease, such a gold standard could be the presence of C. pneumoniae in a coronary atheroma. Identification of the organism in coronary specimens requires invasive testing, and therefore these comparisons are difficult to perform on a large scale. Thus, there are currently no serologic markers that have been proved valid in identifying persons with persistent C. pneumoniae infection, which is a limitation to research efforts in this field.

Our results suggest that a combination of elevated CRP values and chronic infection with C. pneumoniae, H. pylori, CMV or HSV-1 would be helpful when screening for persons at a high risk for CAD. However, the roles of inflammation and infection as potential atherosclerosis risk factors are still unclear and more data and larger prospective studies are necessary to clarify this issue.

ACKNOWLEDGMENTS

This study was supported in part by a grant from Joint Ministry of Health and World Health Organization Regional Office (JPRM) fund (A/C: 02.01.01.01.ACS 2002-03), Bushehr Province Technology and Research Committee and Research Deputy of Bushehr University of Medical Science. We wish to thank Dr. Seyed Rezalamami and Zahra Sanjideh for their kind assistance in field survey.

REFERENCES


Source of Support: Grant from Joint Ministry of Health and World Health Organization Regional Office (JPRM) fund.

Conflict of Interest: None declared.