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 (C. pneumoniae), Herpes simplex virus type 1 (HSV-1), 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 seropositivity to one of the four infectious agents, but 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*

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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.[1]

Numerous studies from various parts of the world have clearly established that CRP predicts future risk for cardiovascular diseases in apparently healthy persons, independent of established risk factors in the majority of studies.[2] In the studies to date, CRP has been shown to predict myocardial infarction, coronary artery disease (CAD) death, stroke, peripheral arterial disease, sudden death, etc.[3] Thus, the Centers for Disease Control and the American Heart Association have issued a statement recommending that patients at intermediate risk of CAD might benefit from measurement of CRP.[4]

This marker of inflammation might, however, be mainly an indicator of classic vascular risk factors (such as smoking and obesity) or of the extent of preexisting disease (since atherosclerosis may be partly an inflammatory lesion).[5] Alternatively, the marker might be an indicator of chronic infective processes possibly correlated with risk of CAD, such as infection by *Chlamydia pneumoniae* (*C. pneumoniae*) or chronic gastric infection with *Helicobacter pylori (H. pylori)*.[6]

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.[6-9] 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. 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.[9]

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

In a multiple-stage stratified cluster random sampling technique, 1,754 persons aged ≥25 years from major ports of Bushehr Province

(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.[10] EKGs were coded on the basis of the Minnesota coding criteria.[11] 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, 29.0% 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 (Minnesota-coding criteria) coronary artery disease. 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 seroprevalences 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), ($P< 0.0001$). 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

Table 1: Clinical characteristics and laboratory values of a random population of the northern Persian Gulf according to electrocardiogram-defined (Minnesota coding) coronary artery disease data

	Normal (n=1532)	*Coronary artery disease (n=222)*	*P value*
BMI, kg/m^2*	27.1 (5.2)	27.9 (5.6)	0.03
Systolic blood pressure, mmHg	124.4 (30.6)	137.3 (67.6)	0.0001
Diastolic blood pressure, mmHg	79.5 (28.3)	86.3 (68.2)	0.008
Total cholesterol, mg/dl	203.8 (46.6)	220.9 (52.9)	0.0001
HDL-cholesterol, mg/dl	44.6 (38.7)	45.6 (26.6)	N.S
Triglyceride, mg/dl	169.1 (102.0)	181.9 (106.4)	N.S
Fasting blood sugar, mg/dl	90.6 (38.4)	103.3 (50.4)	0.0001
C-reactive protein, mg/l**	1.8 (3.8)	2.9 (3.7)	0.0001
Smoking, %	14.4	24.8	0.006
Metabolic syndrome, %	50.8	60.8	0.003
Helicobacter pylori seropositive, %	60.8	67.1	0.04
Cytomegalovirus seropositive, %	93.3	92.8	N.S
Herpes simplex type 1 seropositive, %	86.6	89.3	N.S
Chlamydia. pneumoniae seropositive, %	41.4	35.6	N.S

Values are mean (SD), except for smoking, metabolic syndrome and seropositivity to infectious markers, *BMI indicates body mass index, **Geometric mean (SD).

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 with *C. pneumoniae, H. pylori*, CMV or HSV-1 and electrocardiogram-defined CAD.

Concurrent elevated CRP levels (>10.0 mg/L) and anti-*C. pneumoniae*, *H. pylori*, CMV or HSV-1 IgG antibodies were associated with age- and sex-adjusted prevalence of electrocardiogram-defined CAD in the general population [OR = 1.89 (CI, 1.16–3.08; *P*= 0.01); OR = 2.29 (CI, 1.47–3.57; *P*<0.0001); OR = 1.97 (CI, 1.33–2.92; *P*= 0.001) and OR = 2.12 (CI, 1.42–3.18; *P*<0.0001) respectively). Results remained significant 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

Table 2: Multivariately adjusted odds ratios and 95% CI relating concomitant elevated C-reactive protein levels (>10.0 mg/L) and serum antibodies to infectious agents and electrocardiogram-defined coronary artery disease among the northern Persian Gulf adults*

Coronary artery disease

	Odds ratios*	***CI***	***P value***
Chlamydia pneumoniae + C-reactive protein	1.68	1.24 - 2.59	0.04
Helicobacter pylori + C-reactive protein	1.98	1.26 - 3.13	0.003
Cytomegalovirus + C-reactive protein	1.66	1.10 - 2.49	0.01
Herpes simplex virus type 1+ C-reactive protein	1.79	1.18 - 2.72	0.006

*Adjusted for age, sex and the components of the metabolic syndrome

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 seropositivities 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 covariates, no association was found between positive serostatus against four infectious agents and/or elevated CRP levels and CAD in the northern Persian Gulf general population. However, there was a significant relationship between concomitant elevations of CRP plus seropositivity to *C. pneumoniae, H. pylori*, CMV or HSV-1 and electrocardiogram-defined CAD in our random population sample.

Injury to the vessel wall and the associated inflammatory response are now generally recognized as essential components of atherogenesis.[12] Inflammatory markers are independent predictors of cardiovascular and cerebrovascular events. Large population-based studies, such as the study from the MONICA (MONItoring trends and determinants in cardiovascular disease) Augsberg Center in Germany, the Atherosclerosis Risk in Communities Study, the Women's Heart Study, the Honolulu Heart Study, have also suggested the relation between the levels of CRP and risk of coronary disease.[13] Two recent studies have suggested that serum concentrations of CRP were significant predictors of coronary heart disease even after adjusting for conventional risk factors for coronary heart disease, including serum lipid levels, smoking status and BMI.[14-15] We also demonstrated that serum CRP level was independent of the components of the metabolic syndrome associated with electrocardiogram-defined CAD.

The stimuli that initiate and sustain the inflammatory process have not been fully identified. A candidate trigger of both inflammatory and autoimmune responses is infection, which might be a source of chronic local or systemic inflammation.[9]

We found no significant association between chronic infection and electrocardiogram-defined CAD, beyond traditional cardiovascular risk factors. But in the present study, the significant relationship of concurrent chronic infection and elevated CRP and electrocardiogram-defined CAD, independent to the effects of conventional risk factors, suggests the contribution of chronic low-grade inflammation in chronic infectious process to the atherosclerosis of coronary arteries. It is appealing to speculate that elevation of CRP in *C. pneumoniae, H.*

pylori, CMV or HSV-1 seropositive subjects indicates an active 'smoldering' infectious/ inflammatory process (arteritis?) that accelerates atherothrombotic progression, whereas low CRP in *C. pneumoniae, H. pylori*, CMV or HSV-1 seropositive subjects suggests a resolved or inactive infection.

Kiechl *et al.,* on the basis of prospective results from a large population study, provided solid evidence for the role of common infections in human atherogenesis.[16] They showed that among subjects with chronic infections, atherosclerosis risk was highest in those with a prominent inflammatory response. Mayr *et al.* evaluated cardiovascular risk factors as well as seropositivity to three infectious agents in a population-based study.[17] A significant association between prevalence and severity of atherosclerosis in carotid and femoral arteries and IgA antibodies to *C. pneumoniae* was demonstrated. Odds ratio increased to 4.2 when seropositivity to *C. pneumoniae* was combined with elevated CRP levels. Zhu and colleagues determined the serum CRP levels and the prevalence of serum IgG antibodies to five pathogens (*C. pneumonia*, cytomegalovirus, hepatitis A virus and herpes simplex virus type 1 and 2) and found an association between the total pathogen burden and CRP levels and CAD risk.[18] In a prospective nested case-control study (the Helsinki Heart Study), persistently but not transiently elevated *C. pneumoniae* IC/IgA and hHsp60 IgA antibodies, especially when present together with an elevated CRP level, predicted coronary events.[19] Cytomegalovirus seropositivity and CRP had independent and combined predictive value for mortality in patients with angiographically demonstrated coronary artery disease.[20] Viable *C. pneumoniae* were reported in a substantial portion of carotid artery atherosclerotic plaques and were associated with increased serum CRP.[21] 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 Reza Iamami and Zahra Sanjideh for their kind assistance in field survey.

REFERENCES

1. Black S, Kushner I, Samols D. C-reactive protein. J Biol Chem 2004;279:48487-90.
2. Jialal I, Devaraj S, Venugopal SK. C-reactive protein: Risk marker or mediator in atherothrombosis? Hypertension 2004;44:6-11.
3. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation 2003;107:363-9.
4. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, Criqui M, *et al*. Markers of inflammation and cardiovascular disease-application to clinical and public health practice. Circulation 2003;107:499-511.
5. Danesh J, Whincup P, Walker M, Lennon L, Thomson A, Appleby P, *et al*. Low grade inflammation and coronary heart disease: Prospective study and updated meta-analyses. BMJ 2000;321:199-204.
6. Danesh J, Collins R, Peto R. Chronic infections and coronary heart disease: is there a link? Lancet 1997;350:430-6.
7. Romano Carratelli C, Nuzzom I, Cozzolino D, Bentivoglio C, Paolillo R, Rizzo A. Relationship between Chlamydia pneumoniae infection, inflammatory markers and coronary heart diseases. Int Immunopharmacol 2006;6:848-53.
8. Corrado E, Rizzo M, Tantillo R, Muratori I, Bonura F, Vitale G, *et al*. Markers of inflammation and infection influence the outcome of patients with baseline asymptomatic carotid lesions: A 5-year follow-up. Stroke 2006;37:482-6.
9. Rupprecht HJ, Blankenberg S, Bickel C, Rippin G, Hafner G, Prellwitz W, *et al*. Impact of viral and bacterial infectious burden on long-term prognosis in patients with coronary artery disease. Circulation 2001;104:25-31.
10. Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults. Executive summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285:2486-97.
11. Prineas RJ, Crowe RS, Blackburn H. The Minnesota Code manual of electrocardiographic findings. Bristol: John Wright; 1982.
12. Rose R. Atherosclerosis: An inflammatory disease. N Engl J Med 1999;340:115-26.
13. Corrado E, Novo S. Role of inflammation and infection in vascular disease. Acta Chir Belg 2005;105:567-79.
14. Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A, *et al*. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. N Engl J Med 2004;350:1387-97.
15. Pai JK, Pischon T, Ma J, Manson JE, Hankinson SE, Joshipura K, *et al*. Inflammatory markers and risk of coronary heart disease in men and women. N Engl J Med 2004;351:2599-610.

16. Kiechl S, Egger G, Mayr M, Wiedermann CJ, Bonora E, Oberhollenzer F, *et al*. Chronic infections and the risk of carotid atherosclerosis: Prospective results from a large population study. Circulation 2001;103:1064-70.
17. Mayr M, Kiechl S, Willeit J, Wick G, Xu Q. Infection, immunity and atherosclerosis: association of antibodies to *Chlamydia pneumoniae, Helicobacter pylori* and cytomegalovirus with immune reactions to heat-shock protein 60 and carotid or femoral atherosclerosis. Circulation 2000;102:833-9.
18. Zhu J, Quyyumi AA, Norman JE, Csako G, Waclawiw MA, Shearer GM, *et al.* Effects of total pathogen burden on coronary artery disease risk and C-reactive protein levels. Am J Cardiol 2000;85:140-6.
19. Huittinen T, Leinonen M, Tenkanen L, Virkkunen H, Manttari M, Palosuo T, *et al.* Synergistic effect of persistent *Chlamydia pneumoniae* infection, autoimmunity and inflammation on coronary risk. Circulation 2003;107:2566-70.
20. Muhlestein JB, Horne BD, Carlquist JF, Madsen TE, Bair TL, Pearson RR, *et al*. Cytomegalovirus seropositivity and C-reactive protein have independent and combined predictive value for mortality in patients with angiographyically demonstrated coronary artery disease. Circulation 2000;102:1917-23.
21. Johnston CJ, Messina LM, Browner WS, Lawton MT, Morris C, Dean D. C-reactive protein levels and viable *Chlamydia pneumoniae* in carotid artery atherosclerosis. Stroke 2001;32:2748-52.

Source of Support: Grant from Joint Ministry of Health and World Health Organization Regional Office (JPRM) fund,
Conflict of Interest: None declared.