Chlamydia pneumoniae seropositivity and risk of developing coronary heart disease in Western Saudi Arabia

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ABSTRACT

Objectives: To estimate the seroprevalence of IgG and IgA antibodies against *Chlamydia pneumoniae* (*C. pneumoniae*) among a sample of the Saudi population, and to evaluate whether there is a relationship between seropositivity to chronic infection with *C. pneumoniae* and the manifestation of symptomatic coronary heart disease (CHD).

Methods: We collected 273 sera samples from CHD patients and 273 sera samples from healthy matched controls from the Western region of Saudi Arabia during the period from November 2004 to May 2005. We tested anti-chlamydial IgG and IgA antibodies using enzyme-linked immunosorbent assay technique.

Results: We found 239 (87.5%) patients and 213 (78%) controls positive for *C. pneumoniae* IgG antibodies. However, 58 (21.2%) patients and 55 (23.9%) controls were positive for *C. pneumoniae* IgA antibodies. These results indicate a significant correlation between the presence of IgG antibodies and the development of CHD (p=0.003). Data of this study showed that the presence of IgG antibodies has a 2-fold increase risk in development of CHD. We found no significant correlation between the existence of IgA antibodies and CHD.

Conclusion: Our study indicates that *C. pneumoniae* infection plays an important role in the development of CHD in the Saudi community, emphasizing the importance of developing strategies for prevention and control against this type of bacterial infection. However, we need further study throughout the Kingdom to approve these results in all regions.

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Coronary heart disease (CHD) remains a major cause of human mortality and morbidity worldwide, including the Kingdom of Saudi Arabia.[1] Classical established cardiovascular risk factors, such as cigarette smoking, diabetes mellitus, hypertension, and hypercholesterolemia do not fully explain the temporal and geographical variation in the prevalence of CHD over the past century.[2] Clinical data and animal models suggested that chronic infections (including cytomegalovirus, *Chlamydia pneumoniae* (*C. pneumoniae*), and *Helicobacter pylori*) may also contribute to the pathogenesis of CHD.[3,4] However, an increasing body of evidence implicates *C. pneumoniae* as the most likely etiological factor.[4] The exact possible mechanism by which *C. pneumoniae* may influence cardiovascular risk is unknown. Reports suggest that chronic infection caused by *C. pneumoniae* accompanied by a persistent inflammatory response may contribute to the risk of CHD by increasing the concentrations of acute phase reactants such as fibrinogen and sialic acid, which are predictors of CHD.[5] Saikku et al,[6] in 1988 first demonstrated that elevated serological markers of *C. pneumoniae*

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infection positively associate with CHD. As then, over 30 seroepidemiological studies worldwide demonstrated an association between raised *C. pneumoniae* serological markers such as immunoglobulins (Ig) and immune-complex, and various atherosclerotic vascular diseases.[4,7] Most of these seroepidemiological studies are cross-sectional, case-control studies.[3,4,7-10] They have demonstrated at least a 2-fold increased risk of adverse cardiovascular events with raised serological markers factors.[7] Although, most seroepidemiological studies show a correlation between *C. pneumoniae* and CHD, there are also reports of some negative seroepidemiological studies.[3,8] These negative studies concluded that there was no strong association between *C. pneumoniae* IgG titre and the incidence of CHD, and called for further studies to verify any modest association that may exist. The aim of this study is to estimate the seroprevalence of *C. pneumoniae* among a sample of the Saudi population, and to evaluate whether there is a relationship between seropositivity to chronic infection with *C. pneumoniae* and the manifestation of symptomatic CHD.

Methods. ***Samples.*** We collected 5 ml of venous blood from all Saudi participants in this study and stored the samples in plain tubes. A total of 546 serum samples (from the Saudi population) were collected from 273 patients undergoing therapy for advanced CHD and 273 healthy individuals, during the period from November 2004 to May 2005. The study was carried out at the following hospitals in Makkah and Jeddah cities (Al-Noor Specialist Hospital [560 beds], King Abdul-Aziz Hospital [272 beds], and General King Fahad Hospital [710 beds]). The desired information was obtained from every patient and recorded in the form. The information recorded includes; age, gender, hypertension, diabetes mellitus status, previous myocardial infarction, cholesterol level, CHD family history and smoking habit.

Enzyme-linked immunosorbent assay (ELISA) test. The ELISA automated system (CARO Diagnostic GmbH, Germany) was used to determine the prevalence of IgG and IgA antibodies in both healthy and CHD patients according to the manufacturer recommendation (NovaTec, Germany). The samples were diluted with the sample diluent to obtain a 1:100 dilution.

Statistical analysis. Data were recorded and analyzed using SPSS (version 10). Chi-square test was used to compare frequencies of IgG and IgA antibodies in case and control populations. The odds ratio (OD) for risk of CHD with IgG positive was calculated using Stat 1.0 software. T-test were performed to determine differences between case and control populations regarding age using SPSS (version 10).

Results. T-test revealed no significant difference between case and control populations regarding age.

Coronary heart disease in relation to traditional risk factors. The study showed that hypertension, smoking, obesity, high cholesterol level, and smoking are all statistically correlated with CHD cases in comparison with control cases ($p<0.05$).

Coronary heart disease in relation to IgG level. Sera from 239 CHD patients (87.5%) showed IgG antibody response to *C. pneumoniae*. However, sera tested from the control group showed a response in 213 cases (78%) (**Table 1**). The data obtained showed a highly statistically significant correlation between the existence of IgG antibody against *C. pneumoniae* and CHD ($p=0.003$). The risk of developing CHD in individual displaying IgG positivity was 2 (OR=2, 95% CI=1.25-3.14).

Coronary heart disease in relation to IgA level. Sera from 58 CHD cases showed IgA antibody response to *C. pneumoniae* representing 21.2% from the total samples tested. However, sera tested from the control group showed a response in 65 cases (23.9%) (**Table 1**). The data obtained showed no statistically significant correlation between the existence of IgA antibody against *C. pneumoniae* and CHD ($p=0.540$).

Coronary heart disease in relation to combination of IgA and IgG levels. There was no

Table 1 - Immunoglobulin (Ig) G and IgA levels in relation to coronary heart disease cases and control.

Populations	**Number of patients (%)**			
	IgG positive	**IgG negative**	**IgA positive**	**IgA negative**
Study samples (n=273)	239 (87.5)	34 (12.5)	58 (12.2)	215 (78.8)
Control samples (n=273)	212 (78)	60 (22)	65 (23.9)	208 (76.1)
P-value	0.003		0.540	

statistically significant correlation between the existence of combination of IgA and IgG antibodies level against *C. pneumoniae* and CHD (p=0.650).

Discussion. Important etiological agents implicated for atherosclerosis include chronic infection and inflammation. Among the infectious agents, *C. pneumoniae* is the most possibly etiological factor.[4,9] In this study, we performed ELISA technique to determine the presence of IgG and IgA antibodies to *C. pneumoniae* in both cases of CHD and control sera. The results revealed a statistically significant correlation between the existence of IgG antibody against *C. pneumoniae* and CHD (p=0.003). This result indicates that patients with CHD are significantly more likely than healthy persons to have serologic evidence of past infection with *C. pneumoniae*, possibly involved in the ongoing process of inflammation (OR=2). We found no statistically significant correlation between either the existence of IgA antibody or a combination of both IgG (p=0.540) and IgA (p=0.650) against *C. pneumoniae* and CHD. The positive correlation between the existence of IgG antibody against *C. pneumoniae* and CHD found in this study agrees with several previous international studies.[4,9-23] Our results are also in agreement with previous studies that found atherosclerotic patients are more frequently infected with Cytomegalovirus (CMV) or *C. pneumoniae* or both, in Saudi patients using the PCR technique. However, both studies included only a small sample size (43 and 17 cases).[24,25]

In contrast to our study, several studies found no association linking CHD and *C. pneumoniae* IgG antibodies.[3,7,8,26-31] We can explain the differences in the results observed in our study compared with these studies by various reasons including;[22] 1. The use of different serological methods in different studies and the relation of the different results to the choice of methods. 2. The use of different titer limits in different studies, and most studies classify individuals as seropositive or seronegative based on low titer limits of IgG or IgA; low titers will not distinguish between passed and persistent infection. 3) Ethnic variations with different stages of atherothrombotic disease may account for the diverging results. The non-significant correlation between the existence of anti chlamydial IgA antibody, and CHD achieved in our study is in agreement to those found in previous studies.[15,27,31-34] A recent study found that 57% were seropositive for IgA in the cases and 53% in the controls tested.[31] However, in contrast to our study, several studies found a strong association between CHD and the presence of *C. pneumoniae* IgA antibodies.[23,30] A recent study found that 67% were seropositive for IgA in the cases and 29% in the controls tested.[23] They explained this by the fact that IgA antibodies have a short half life, which is only 5-7 days and they usually decline rapidly following treatment of the chlamydia infections while IgG antibodies persist for long periods.[23,24]

In conclusion, we should consider the significant correlation between *C. pneumoniae* IgG and risk of developing CHD achieved by this study in order to develop potential targeted antimicrobial treatments and possibly vaccines for *C. pneumoniae* infection aiming to decrease the risk of developing CHD. However, we should perform a national survey on the association between *C. pneumoniae* and CHD throughout the Kingdom before applying these preventive actions, as no such national data are currently available.

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