

CHLAMYDIA PNEUMONIAE SEROPREVALANCE IN SAUDI CORONARY ARTERY DISEASE PATIENTS

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CHLAMYDIA PNEUMONIAE (*C. PNEUMONIAE*) IS ONE OF THE LEADING CAUSES of acute respiratory tract infections in humans. Recent studies suggest an association between *C. pneumoniae* and coronary artery disease (CAD). A case-control study was conducted to investigate the association between *C. pneumoniae* seropositivity as measured by Immunoglobulin G (IgG) antibody using enzyme linked immunosorbent assay (ELISA) and both CAD and cardiovascular risk factors among Saudi patients. Results showed that *C. pneumoniae* sero-prevalence was 14% among CAD patients. Patients were found to be more chronically exposed to infection and reinfection with *C. pneumoniae* than control as evaluated by the presence of IgA and IgM antibodies. These results did not support the association between *C. pneumoniae* and CAD because of the mismatched cases and controls.

Chlamydia pneumoniae (*C. pneumoniae*) causes acute respiratory tract infections in both adults and children. In 1988 Saikku et al. from Finland provided evidence for association between chronic *C. pneumoniae* infection and coronary heart disease (CAD). This was shown by elevated antibodies or chlamydia lipopolysaccharide containing immune complexes in 50% to 60% of patients with acute myocardial infarction or CAD.¹ This was followed by several studies that provided further evidence of such an association. These studies relied on serology as well as the detection of the organism or immune complexes containing chlamydial lipopolysaccharide in more than 60% of atheromatous plaques using molecular biology studies, electron microscopy or immunocytochemistry.^{2,3,4}

The studies which have been conducted in Saudi population were concerned about the established risk factors for developing CAD including diabetes mellitus, hypertension, smoking, serum lipids and age.^{5,6} To our knowledge there has been no previous study on *C. pneumoniae* and its association with CAD among Saudi population.

The purpose of this study was to investigate the association between chronic *C. pneumoniae* infection and the development of CAD. The investigation is a case-control study comparing the seroprevalence of *C. pneumoniae* in a group of Saudi patients, with angiographically documented CAD with a control group. The enzyme-linked immunosorbent assay test (ELISA)⁷ was used in this study.

Subjects and Methods

Subjects

A case-control study was conducted among Saudi patients (n = 100) undergoing coronary angiography at King Khalid University Hospital, Cardiac Catheterization Laboratory between December 1996 and July 1997. Patients had at least one coronary artery lesion occupying at least 50% of the luminal diameter. 100 patients of both sexes aged between 35 and above were studied. Controls were (n = 100) asymptomatic Saudi blood donors with the same age range as patients. All subjects were interviewed by means of a structured questionnaire for general demographic details, current living circumstances, smoking habits, number of children at home, exercise practiced and past medical history. This included diabetes mellitus, hypertension, past and family history of ischemic heart disease or any other disease. History

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of myocardial infarction and symptoms of angina were obtained according to the Rose angina questionnaire.⁸ Patients were asked about frequent flu-like illness in order to get a probable history of recent chlamydia infection. Blood pressure and weight in kilogram were measured for both patients and controls.

Methods

Twenty mL of fasting blood was withdrawn from patients at the time of catheterization and serum was tested for plasma fibrinogen and lipid profile. This included cholesterol, triglyceride, high-density lipoprotein (HDL) and low-density lipoprotein (LDL). The serum was also tested for specific immunoglobulin, IgG, IgM, and IgA antibodies to *C. pneumoniae* by a commercial ELISA test (Invitro Diagnostika GmbH, Germany). This test utilizes specific *C. pneumoniae* antigen. For the controls, 10 mL of non-fasting serum was tested for *C. pneumoniae*, only using ELISA test was for the patients. The ELISA tests were performed

Antibody results were presented as negative, positive and high positive. For *C. pneumoniae*, negative results for IgG, IgA and IgM were < 44, < 48, and < 65 U/mL respectively. A positive test was at a titre between 75 to 100 (IgG), 81 to 110 (IgA) and 91 to 120 (IgM) U/ml. Titres of > 100, > 110, and > 120 U/mL for IgG, IgA and IgM, respectively, were considered as high positive. For reason of easy interpretation, the positive and high positive results for IgG, IgM and IgA were included as positive.

Statistical Methods

Mann-Whitney test was used to compare geometric means for patients and controls. Two-tailed probability testing estimated the significant association between *C. pneumoniae* seropositivity and the development of CAD among patients and controls. *P*-value was calculated by Chi-square.

Results

The demographic characteristics and risk factors for patients and controls are shown in Table 1. Ninety nine percent (99%) of the control group were men and the patients group comprised 80 males (80%) and 20 females (20%). Patients were more likely to be older than controls (50% were over 60

Table 1. Demographic characteristics and risk factors for patients and control.

| Characteristics | Cases (no = 100) (no = %) | Control (no = 100) (no = %) |
|--------------------------------|------------------------------|--------------------------------|
| Sex: | | |
| Male | 80 | 99 |
| Female | 20 | 1 |
| Age ¹ (years): | | |
| 35 - 39 | 5 | 50 |
| 40 - 45 | 5 | 27 |
| 46 - 50 | 11 | 11 |
| 51 - 55 | 10 | 8 |
| 56 - 60 | 19 | 4 |
| > 60 | 50 | 0 |
| Marital status: | | |
| Married | 95 | 98 |
| Unmarried | 2 | 2 |
| Unknown | 3 | 0 |
| No. of children: ² | | |
| < 5 | 42 | 41 |
| 5 - 10 | 34 | 45 |
| 11 - 15 | 18 | 12 |
| > 15 | 6 | 2 |
| Smoking: | | |
| Current | 30 | 31 |
| Ex | 10 | 17 |
| Non | 70 | 52 |
| History of heart disease: | 36 | 0 |
| History of diabetes: | 36* | 0 |
| History of high blood pressure | 39 | 3 |
| Exercise activity | 6 | 42 |
| Flu like illness | 6 | 4 |
| Weight/Kg: | | |
| < 70 | 43 | 18 |
| 70-75 | 19 | 15 |
| 76-80 | 11 | 11 |
| 81-85 | 14 | 14 |
| 86-90 | 5 | 12 |
| > 90 | 8 | 30 |

¹mean age for patients = 59 years

mean age for control = 40 years

²mean = 6 (for patients and control)

* Significant value = < 0.05 (Chi-square)

years, whereas 50% of controls were less than 40 years old).

In this study, the overall antibody seropositivity for *C. pneumoniae* IgG was 17% ,14% of which were among patients, ($P = < 0.001$). Seven percent of the patients over 60 years of age had positive result. None of the control had positive IgG titres above the age 56. None of the females had a



Table 2. Prevalence of *C. pneumoniae* IgA and IgM among patients and control with negative and positive IgG. (n = %).

| | Patients | | | Control | | |
|----------|------------|-----|---------|-------------|------|----------|
| | IgG/IgM | IgA | P-value | IgG/IgM | IgA | P-value* |
| Negative | 86% to 96% | 97% | < 0.05 | 97% to 100% | 100% | 0.8 |
| Positive | 14% to 4% | 3% | < 0.05 | 3% to 0% | 0% | - |

*Significant value = < 0.05

detectable antibody results. A significant association ($P = < 0.05$) was found between history of diabetes and IgG seropositivity among patients (36% of patients had a history of diabetes mellitus). Three percent of control had IgG seropositivity for *C. pneumoniae* with no significant association with any of the risk factors.

The association between *C. pneumoniae* IgG and other conventional risk factors for CAD in patients were raised triglycerides > 2.27 mm/L and fibrinogen > 4 g/L ($P = < 0.000$) and < 0.001 respectively).

Table 2 presents the prevalence of *C. pneumoniae* IgM and IgA among patients and controls with negative, and positive IgG results. Three percent of the patients had positive IgA, indicating the persistence of active infection in patients. Four percent (4%) of patients had positive IgM, indicating recent or reactivation of infection, ($P = < 0.05$). IgM and IgA were not detected in any control.

Discussion

infection) was developed to explain the initiation of atherosclerosis.⁹ A previous *C. pneumoniae* infection is found to be a significant risk factor for the development of CAD.^{3,4,10,11} In this study at KKHU, Saudi Arabia, the findings did not show a similar relationship. A limitation of this study is the dissimilarity between the mean age of the patients and the control group. This was due to the fact that most of those who come for blood donation were less than 40 years of age. Age has a positive effect on the rate of seropositivity for *C. pneumoniae* because of prolonged exposure and high recurrence rate in older people. The overall prevalence of *C. pneumoniae* IgG in this study is 8.5%. In Finland, Shor et al.¹² found a prevalence of *C. pneumoniae* antibodies in 50% of patients with CAD and 68% of patients with acute myocardial infarction but in only 17% of the control group.

The slightly lower prevalence of *C. pneumoniae*

antibodies among Saudi population compared to Western countries could be explained by geographical differences. Saikku¹³ reported that infection is more prevalent near the equator, but in the Kingdom of Saudi Arabia the weather is hot and dry most of the year.¹³ A more or less steady endemic situation prevails in southern latitudes and *C. pneumoniae* tends to be an important early childhood pathogen in tropical urban slums.

History of diabetes mellitus and triglycerides of > 2.27 were significantly associated with high positive IgG and fibrinogen of > 4g/L, with positive IgG titres respectively, among patients with CAD ($P = < 0.05$, < 0.000, < 0.001 respectively). Only history of diabetes was correlated with seropositivity. However, doing fasting blood glucose test on one occasion does not correlate well with the diabetic status of the patient. The significant correlation with high triglycerides is also compatible with our result since triglycerides couple with diabetes. In the Helsinki heart study Saikku et al.¹³ reported that the prevalence of dyslipidemia (the ratio of total cholesterol to HDL-cholesterol) of more than 6 was negatively associated with high level of *C. pneumoniae* antibodies.

The observation that 3% of our patients have positive IgA response but none in the control group suggests that those patients had persistence of active infection. Mendall et al. suggested that the more strongly positive IgG titre the more likely there is to be a detectable IgA response.⁴ Melnick et al. in a cross-sectional study provided evidence that prior TWAR infection is strongly related to asymptomatic cardiac atherosclerosis.¹⁴ Therefore, *C. pneumoniae* IgG measurement can be used to predict and so prevent cardiac attack.

Regarding IgM antibodies, 4% of the patients with IgG antibodies had positive IgM ($P = < 0.05$) compared to none in the control group. This indicates recent infections with *C. pneumoniae* in these patients. However, Melnick et al. did not find IgM antibodies to TWAR and recent infection in her study.¹⁴



Conclusion

We conclude that *C. pneumoniae* is prevalent in Saudis and that patients are more chronically exposed to infection and reinfection than controls. We recommend conduction of a countrywide prospective studies with matched age between patients and control and eradication trials with the appropriate antibiotics in order to confirm and evaluate the causal relationship between *C. pneumoniae* and CAD.

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