

Research Article

Association of Chlamydia pneumoniae IgG and IgA antibody in coronary artery disease

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ABSTRACT

Background: Cardiovascular disease, resulting from atherosclerosis, is a leading cause of global morbidity and mortality. Classical risk factors explain much of the attributable risk for cardiovascular events, but other risk factors for the development and progression of atherosclerosis, which can be identified, may be important therapeutic targets. Infectious agents, such as Chlamydia pneumoniae, have been proposed as contributory factors in the pathogenesis of atherosclerosis. The present study was conducted to determine the seroprevalence of C. pneumoniae antibodies and to study the association of chronic C. pneumoniae infection with Coronary Artery Disease (CAD).

Methods: The study group included 90 angiographically proven CAD patients and age and sex matched 90 normal coronaries as control group. With total aseptic precaution 3 ml blood was collected. Enzyme linked immunosorbent assay was performed for all subjects to detect the presence of IgG and IgA antibodies to Chlamydia pneumoniae (Cp).

Results: IgG and IgA Cp antibodies were detected in 67.8% and 58.9% CAD patients compared to 45.6% and 11.1% controls. IgG + IgA Cp antibodies were detected in 88.9% CAD patients when compared to 50.0% controls. Seroprevalence of IgG and IgA Cp antibodies were high among CAD patients compared to controls and was found statistically significant. A significant presence of Chlamydia pneumoniae antibodies was detected in smokers, diabetes mellitus, hypertension, and dyslipidemia.

Conclusion: In the present study, the seroprevalence of IgG and IgA Cp antibodies was found to be higher in CAD patients compared to controls. The present study supports the association between Chlamydia pneumoniae infection and Coronary artery disease.

Keywords: Chlamydia pneumoniae, Coronary artery disease

INTRODUCTION

Coronary artery disease is a major cause of morbidity and mortality in humans and is predicted to be the leading cause of death in the world.¹ The major pathologic process underlying the cardiovascular disease is atherosclerosis, which progresses over many years, influenced by individual's genetic constitution and important "environmental" risk factors.² For decades research on the pathogenesis of vascular disease has been focused on classical risk factors, including hyperlipidemia,

hypertension, smoking, diabetes, sex, age, and familial history. However, not all cases can be explained by these well-defined risk factors.³ Therefore, the search for novel potential risk factors is continuing.⁴

Various infectious pathogens, including Helicobacter pylori, Cytomegalovirus, Herpes simplex virus, and C. pneumoniae, have been considered as potential risk factors for vascular diseases.^{5,6} Chlamydia pneumoniae, a gram negative obligate intracellular bacterium, ubiquitous respiratory pathogen can initiate inflammation and lead to

chronic infection. There are evidences to show that this may contribute to the development of vascular disease.^{5,6} After respiratory tract infection, *C. pneumoniae* can reach vascular tissue via infected leukocytes, where it can infect cells associated with atherosclerosis like endothelial cells, macrophages and smooth muscle cells. Chlamydial lipopolysaccharide and Chlamydial heat shock protein 60 kd (cHsp60) may contribute to atherogenesis in several ways.⁶ Lipopolysaccharide mediates ingestion of Low-Density Lipoprotein (LDL) by macrophages infected with *C. pneumoniae*, leading to the formation of foam cells, the characteristic cells of early atherosclerosis. cHsp60 mediates oxidation of lipoproteins and also cause pro inflammatory activation which promotes atherogenesis.⁷

These associations are determined by seroepidemiologic observations, case reports, isolation or direct detection of the organism in specimens, successful response to anti chlamydial antibiotics or combination of these methods.⁸ It is reported that India have the highest risk of CAD and the prevalence of CAD in India has recently been estimated to be 11%.³ Serological studies indicate a prevalence rate of *C. pneumoniae* 40-70% in many countries.⁹

Hence present study was conducted to determine the seroprevalence of *C. pneumoniae* antibody and association of chronic infection with CAD.

METHODS

A case control study was conducted in the Department of Microbiology and Sri. Jayadeva institute of cardiovascular science and research, K. R. hospital premises, Mysore, India, from Jan 2012 to Dec 2012. The study comprised of case group consisting of 90 subjects who had Coronary Artery Disease (CAD) that defined by presence of at least one coronary artery lesion occupying at least 50% of luminal diameter in coronary angiography. The control group consisted of 90 subjects of both sex without coronary artery disease in angiography were matched for age and sex. Exclusion criteria were patients with malignancy, connective tissue disorders and immune system deficiency.

A detailed history was taken with reference to name, age, sex, occupation, chest pain, breathlessness, H/o diabetes mellitus (FBS>126 mg/dl), hypertension [Systolic BP >140 mg and Diastolic Blood Pressure (DBP) >90 mg], smoking, dyslipidemia (Total cholesterol >200 mg/dl), family H/o coronary artery disease and presence of other systemic disease were documented on a predesigned proforma.

Under aseptic precautions 3-5 ml of venous blood was collected from the case and control group and was added to sterile non- heparinized tubes. Serum was separated and transferred to a clean vial. All the serum samples were subjected for the detection of IgG and IgA

antibodies to *Chlamydia pneumoniae* (Cp) by Enzyme Linked Immune-Sorbant Assay (ELISA) using (Novatec kit, Germany) and the test was conducted as per manufacturer's instructions. The lipid profile was analyzed for all the subjects. The results obtained were analysed statistically using Chi square test.

RESULTS

A total of 180 study subjects, comprising of 90 CAD patients and 90 controls were included. Among them 70 (77.8%) were males and 20 (22.2%) were females.

Table 1: Age wise distribution of study subjects.

Age group (years)	CAD patients n (%)	Controls n (%)
40-49.5	24 (26.7%)	24 (26.7%)
50-59.5	37 (41.1%)	37 (41.1%)
60-69.5	26 (28.9%)	26 (28.9%)
70-79.5	3 (3.3%)	3 (3.3%)
Total	90 (100%)	90 (100%)

Majority of subjects 87 (96.7%) were in the age group range 40-70 years and coronary artery disease was commonly seen in 50-59.5 years (41.1%) age group (Table 1).

Table 2: Distribution of selected risk factors among patients and controls.

Risk factors	CAD patients n (%)	Controls n (%)	P value
Smoking	42 (46.7%)	23 (25.6%)	0.003 (S)
DM (diabetes mellitus)	37 (41.1%)	22 (24.4%)	0.017 (S)
HTN (hypertension)	42 (46.7%)	16 (17.8%)	0.000 (HS)
Dyslipidemia	67 (74.4%)	40 (44.4%)	0.000 (HS)

S - Significant, HS - Highly significant

Among the various risk factors hypertension and dyslipidemia showed significant association with a significant statistical difference (Table 2).

Table 3: Seroprevalence of IgG, IgA, and IgG+IgA Cp antibodies among study subjects.

Anti Cp antibodies	Study group		P value
	CAD patients (n=90)	Controls (n=90)	
	n (%)	n (%)	
IgG	61 (67.8%)	41 (45.6%)	0.011 (S)
IgA	53 (58.9%)	10 (11.1%)	0.004 (S)
IgG+IgA	80 (88.9%)	45 (50.0%)	0.000 (HS)

There was statistically significant difference among the different study groups as regarding the prevalence of *C. pneumoniae* (Cp) - specific IgG, IgA, IgG and IgA

antibodies ($P < 0.05$) as shown in Table 3 with an Odds Ratio (OR) = 2.52 for IgG Cp and OR = 4.9 for IgA Cp antibodies.

Table 4: Comparison of IgG, IgA and IgG and IgA Cp antibody among various risk factors.

Risk factors	IgG Cp antibody n (%)		IgA Cp antibody n (%)		IgG + IgA Cp antibody n (%)	
	Positive	P value	Positive	P value	Positive	P value
Smoking	44 (67.7%)	0.025 (S)	36 (55.4%)	0.000 (HS)	57 (87.7%)	0.000 (HS)
Diabetes mellitus (DM)	39 (66.1%)	0.074 (NS)	29 (49.2%)	0.005 (S)	47 (79.7%)	0.030 (S)
Hypertension (HTN)	40 (69.0%)	0.022 (S)	30 (51.7%)	0.001 (S)	47 (81.04%)	0.020 (S)
Dyslipidemia	72 (67.3%)	0.000 (HS)	55 (51.4%)	0.000 (HS)	90 (84.1%)	0.000 (HS)

C. pneumoniae IgG, IgA, IgG and IgA seropositivity and their association with various risk factors were assessed, association of smoking and dyslipidemia was found to be highly significant (Table 4).

DISCUSSION

Cardiac disease has known as the most common cause of mortality and morbidity in the world. Coronary artery atherosclerosis is considered as the main cause of cardiac disease.³

Atherosclerosis is a common pathological process associated with acute and chronic coronary heart disease and ischemic stroke. Inflammation seems to be a key event in the development and progression of atherosclerosis. *C. pneumoniae* has been strongly linked to and implicated in the inflammatory and procoagulatory component of atherosclerosis.¹⁰ Chlamydia pneumoniae serology consists of various markers that help in detecting a previous or an on-going infection.¹¹ In this study, the presence of IgG and IgA antibodies against *C. pneumoniae* in sera of CAD patients and controls was determined by a commercial qualitative ELISA method.

Among the various risk factors assessed, in our study *C. pneumoniae* seropositivity (IgG and IgA) was high among smokers than among non-smokers (67.7%, 55.4%). The results observed in our study were in accordance with the previous findings.^{12,13} These results suggest that smoking increases susceptibility to Cp infection. Most of the proposed proatherogenic actions of smoking, such as interference with blood coagulation, induction of endothelial dysfunction, and promotion of lipid peroxidation, reverse themselves shortly after cessation of smoking. Such a pathomechanism may be relevant to the development of vessel pathology among smokers because of the facilitating effects of smoking on the manifestation of various types of persistent infectious illness.¹

In the present study, dyslipidemia was significantly associated with Chlamydia pneumoniae (IgG and IgA) seropositive subjects when compared with seronegative individuals, indicating a possible indirect effect of infection with CAD. Similar observations are reported in other studies which are consistent with ours.^{14,15} Chlamydia induces production of several cytokines leading to altered lipid metabolism, accumulation of serum triglycerides and a decrease in HDL and in chronic Chlamydial infection, this would be compounded by the continuous production of pro-inflammatory cytokines.¹⁴

In our study a significantly higher seroprevalence of Chlamydia pneumoniae IgG (67.8%) was observed in CAD patients compared to controls (45.6%) with a significant odds ratio of 2.52 was found (Table 3). The possession of IgG antibody to Chlamydia pneumoniae denotes either previous exposure (with antibody persistence) or chronic or latent infection.¹⁶ The results obtained in our study are consistent with other studies where in, a study by Jaremo and Richter (2004) who studied Chlamydia pneumoniae IgG and the severity of coronary atherosclerosis has presented that the more serum level of IgG, the more coronary artery involvement.¹⁷ In another study by Lt col Agarwal et al. association of CAD with chronic Chlamydia pneumoniae infection were assessed and they found that Chlamydia pneumoniae IgG antibodies was 61% in CAD group as compared to 38% in control group.⁸

Similarly, in present study a significantly higher seroprevalence of Chlamydia pneumoniae IgA (58.9%) was observed in CAD patients compared to controls (11.1%) with a significant odds ratio of 4.9 was found (Table 3). The persistence of IgA antibody has been thought to reflect chronicity, because the half-life of IgA antibody is shorter than that of IgG antibody,¹⁸ while patients having both IgG and IgA antibody may reveal chronicity or persistent active infection¹ and the presence of IgA is more indicative of persistent antigenic stimulation by an on-going infection.¹⁹ The results

observed in our study are in accordance with other studies. A Study done by Mazzoli et al. (1998) showed a high prevalence rate of high titre of IgG (82%) and IgA (71%) anti *C. pneumoniae* antibodies in CAD patients compared to controls (34%, 14.9%).²⁰ However they used two different tests to detect anti-*C. pneumoniae* antibodies: recombinant enzyme immunoassay antilipopolsaccharide antibodies and a reference microimmunofluorescence (MIF) test. Leowattana et al. (2000) investigated the relationship between the presence of IgG, IgA of *Chlamydia pneumoniae* in angiographically diagnosed CAD patients. They found 73.7% of CAD patients were IgG and 54.3% of CAD patients were IgA positive when compared with healthy controls and the results were statistically significant in the higher odds ratio.²¹ Our results are also consistent with the study done by Masato Nishimura et al. which showed that seropositivity for *C. pneumoniae* IgA (75.5%) was found in patients with coronary stenosis than in patients without coronary stenosis (16.9%).²²

It is difficult to compare the results of most seroepidemiologic studies on the association between *C. pneumoniae* and CHD, because these are done on distinct populations, used various cut-off titers and antibody fractions for *C. pneumoniae* seropositivity. In our study we have used ELISA for detection of *C. pneumoniae* antibodies but most of the studies have used MIF to detect *C. pneumoniae* antibodies, and the interpretation of the results should be commenced by expert microscopists and therefore may be subjected to bias. Recently increasing body of evidence links infections to atherosclerosis. Therefore, it is hypothesized that infections could interact with other risk factors of vascular disease, enhancing the endothelial damage and the production of atherosclerotic plaques.²³

CONCLUSION

The results of the present study suggest that there is a relationship between *Chlamydia pneumoniae* infection and Coronary Artery Disease (CAD). However, these studies do not establish a causal relationship for the development of CAD. It is also possible that CAD risk factors and CAD may predispose the host to develop and maintain the chronic *Chlamydia pneumoniae* infection. To establish the relationship between CAD and *Chlamydia pneumoniae* complete and comprehensive studies may be necessary, which may help in further interventions such as vaccination and antibiotic therapy, in order to reduce the disease or recovery of patients.

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