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Original Research Article

Serological Evidence of Chronic Chlamydia Pneumoniae Infection in Coronary Artery Disease

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Abstract:

Background and Objectives: Cardiovascular disease, resulting from atherosclerosis, is a leading cause of global morbidity and mortality. Genetic predisposition and classical environmental risk factors explain much of the attributable risk for cardiovascular events in populations, but other risk factors for the development and progression of atherosclerosis, which can be identified and modified, may be important therapeutic targets. Infectious agents, such as *Chlamydia pneumoniae*, have been proposed as contributory factors in the pathogenesis of atherosclerosis. Hence 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.

Methodology: The study was performed on patients attending the outpatient department as well as on patients admitted for coronary angiogram at PMCH Patna, over a period of one year in the department of Microbiology, PMCH Patna. 3 ml of blood was collected aseptically from 90 angiographically proven coronary artery disease (CAD) patients and 90 number of age and sex matched healthy control subjects. Enzyme linked immunosorbant assay was performed for all the 90 CAD patients and 90 controls to detect the presence of IgG and IgA antibodies to *Chlamydia pneumoniae* ESR, CRP, Blood glucose, lipid profile was analyzed for all the cases and controls.

Conclusion: In the present study, the seroprevalence of IgG and IgA Cp antibodies was found to be higher in coronary artery disease patients compared to controls and this difference was statistically significant. The present study supports the association between *Chlamydia pneumonia* infection and coronary artery disease.

Keywords: *Chlamydia pneumoniae,* Coronary Artery Disease.

Introduction

Coronary artery disease (CAD) is a major cause of morbidity and mortality in humans and is predicted to be the leading cause of death in the world. [1] Atherosclerosis the pathological basis of coronary artery disease (CAD) and ischemic stroke is the commonest cause of death and disability in the world [2] .There is a large amount of factual data that suggests that *Chlamydia pneumoniae* plays a role in atherosclerosis. [1]

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. Therefore, the search for novel potential risk factors is continuing.

A central role for inflammation in atherogenesis has been established. Current evidence indicates that inflammatory processes are implicated in the initiation and the evolution of the atherosclerotic process. [3,4] The initial step in atherosclerosis is probably endothelial dysfunction, which may be caused by a risk factor or a combination of several risk factors. [4] Various infectious pathogens, including *Helicobacter pylori*, Cytomegalovirus, Herpes simplex virus, and *C.pneumoniae,* have been considered as potential risk factors for vascular diseases. The hypothesis that infection might play a role in the development of vascular diseases supposes that infectious agents, such as *C. pneumoniae* (Cp), initiate and progress inflammation, which may contribute to the development of vascular disease. [5,6,7] After respiratory tract infection, *C. pneumoniae* can reach vascular tissue via infected leukocytes, where it can infect cells associated with atherosclerosis 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, which become atherogenic . cHsp60 may also cause pro inflammatory activation, which promotes atherogenesis . It has been suggested that cHsp60 may induce immunological cross-reaction with autoantigens such as human Hsp60 leading to antibody-mediated endothelial cytotoxicity. [7] Moreover, infected atheroma associated cells, such as endothelial cells, seem to produce inflammatory cytokines and express leukocyte adhesion molecules. Endothelial infection may stimulate smooth muscle cells proliferation, whereas infection of macrophages and smooth muscle cells induces production of inflammatory cytokines. [6] It has been suggested that *C. pneumoniae* may cause impaired arterial relaxation and endothelial dysfunction. [8] Moreover, a role for *C. pneumoniae* infection in plaque destabilization has been postulated; it may promote the secretion of matrix-degrading metalloproteinases that destabilize the atherosclerotic plaque. [6] Since the reported association between *C. pneumoniae* and coronary artery disease in 1988, the theory of *C. pneumoniae* as a cause of vascular disease has received considerable attention. [9]

Objectives

To determine the seroprevalence of *Chlamydia pneumoniae* antibodies in angiographically demonstrated coronary artery disease.

To study the association between Coronary artery disease and *Chlamydia pneumoniae* infection.

Material and Methods

The study was performed on patients attending the outpatient department as well as on patients admitted for coronary angiogram at PMCH Patna, over a period of one year. in the department of Microbiology, PMCH Patna. 3 ml of blood was collected aseptically from 90 angiographically proven coronary artery disease (CAD) patients and 90 number of age and sex matched healthy control subjects. Enzyme linked immunosorbant assay was performed for all the 90 CAD patients and 90 controls to detect the presence of IgG and IgA antibodies to *Chlamydia pneumoniae* . ESR, CRP, Blood glucose, lipid profile was analyzed for all the cases and controls.

A total of 90 angiographically proven coronary artery disease patients were enrolled in the study as cases and were compared with 90 number of age and sex matched healthy control subjects.

Inclusion Criteria

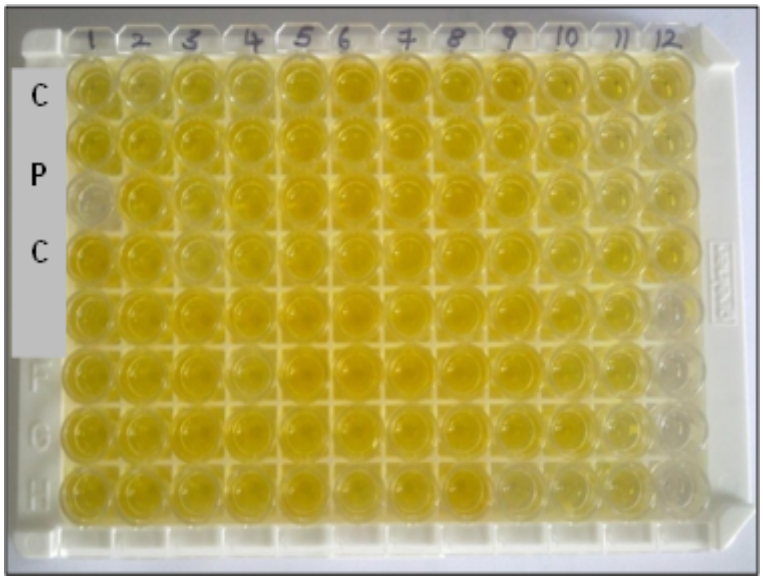
Patients who had at least one coronary artery lesion occupying at least 50% of the luminal diameter on coronary angiography were included in the study.

Healthy controls were selected from the OPD of the hospital who came for routine health check-up and had no symptoms and clinical evidence of atherosclerotic disease including a negative stress test.

Exclusion Criteria

Patients with history of malignancy, connective tissue disorders, immune system deficiency, use of immunosuppressive drugs and other chronic illness were excluded from the study.

A detailed history was taken with reference to name, age, sex, occupation, chest pain, breathlessness, h/o Diabetes mellitus, Hypertension, obesity, smoking, dyslipidemia, Family h/o coronary artery disease and presence of other systemic disease were documented on a predesigned proforma.
About 3 to 5 ml of blood was collected in a sterile vial following venepuncture with all aseptic precautions. Blood was allowed to clot at room temperature for 30-45 minutes. This was centrifuged at 3000 rpm for 10 minutes. The resulting supernatant was immediately transferred to a clean vial using pasteur pipette. Serum sample were stored at – 20^0C. Enzyme linked immunosorbant assay was performed for all the 90 angiographically proven coronary artery disease patients and 90 controls to detect the presence of IgG and IgA antibodies to *Chlamydia pneumoniae* as per manufacturer‟s instructions. ESR, CRP, Blood glucose, lipid profile was analyzed for all the cases and controls.



- Cut off, PC – Positive control, NC - negative control, and test samples S1, S2, etc

Results

Table 1: Age wise distribution of CAD patients and controls

Age Group (Years)	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%)

Among 90 CAD patients and 90 controls studied, coronary artery disease was commonly seen in 50-59.5 yrs (41.1%) age group.

Table 2: Sex wise distribution of CAD patients and controls

Sex	Patients n (%)	Controls n (%)
Male	70 (77.8%)	70 (77.8%)
Female	20 (22.2%)	20 (22.2%)
Total	90(100%)	90(100%)

Among 90 CAD patients and 90 controls 70(77.8%) were Males and 20(22.2%) were Females.

Table 3: Comparison of CAD Patients and controls based on various risk factors

Risk Factors	Patients n (%)	Controls n (%)	P Value
Obesity	12 (13.3%)	10 (11.1%)	0.649 (NS)
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)
Family h/o CAD	7 (7.8%)	15 (16.7%)	0.069 (NS)

P value; S-Significant, HS-Highly significant, NS- Not significant

Among the various risk factors studied between cases and controls, association of smoking, Diabetes mellitus, Hypertension and dyslipidemia were found to be significant with a statistically significant P value of (0.003, 0.017, 0.000, 0.000).

Table 4: Distribution of CAD patients by clinical diagnosis

Clinical diagnosis	Number	%
Effort angina(EA)	21	23.3%
Unstable angina(UA)	19	21.1%
Myocardial Infarction(MI)	50	55.6%
Total	90	100%

P Value – 0.000-significant

Out of 90 CAD patients 21(23.3%) had EA, 19(21.1%) had UA and 50(55.6%) had MI. In the present study majority of CAD patients presented with myocardial infarction (55.6%).

Table 5: Seroprevalence of IgG Cp antibody among study subjects

Study Group	IgG Cp antibody, n (%)		Total n (%)
	Positive	Negative	
Patients	61(67.8%)	29(32.2%)	90(100%)
Controls	41(45.6%)	49(54.4%)	90(100%)
	102(56.7%)	78(43.3%)	180(100%)

OR-2.52-significant, P value 0.011-significant

Out of 90 CAD patients 61(67.8%) and among 90 controls 41(45.6%) were positive for IgG Cp antibody. Hence the prevalence of IgG Cp antibody positivity was high among CAD patients compared to controls and the difference between them was statistically significant. Further a statistically significant odds ratio of 2.52 was found.

Table 6: Seroprevalence of IgA Cp antibody among study subjects

Study Group	IgA Cp antibody, n (%)		Total n (%)
	Positive	Negative	
Patients	53(58.9%)	37(41.1%)	90(100%)
Controls	10(11.1%)	80(88.9%)	90(100%)
	63(35.0%)	117(65.0%)	180(100%)

OR-4.9- significant, P value-0.0043-significant

Out of 90 CAD patients 53(58.9%) and among 90 controls 10(11.1%) were positive for IgA Cp antibody. Hence the prevalence of IgA Cp antibody positivity was high among CAD patients compared to controls and the difference between them was statistically significant. Further a statistically significant odds ratio of 4.9 was found.

Table 7: Comparison of Lipid profile with IgG + IgA Cp antibodies

Lipid Profile (Mean ± SD)	IgG + IgA Cp antibodies	P Value	
	Positive	Negative	
Total cholesterol	196.40 ± 39.59	184.60 ± 33.26	0.055 (S)
Triglycerides	190.67 ± 61.66	164.15 ± 51.55	0.006 (S)
High density lipoprotein (HDL)	35.80 ± 6.43	40.09 ± 6.14	0.000 (HS)
Low density lipoprotein (LDL)	125.14 ± 36.28	110.41 ± 26.76	0.008 (S)
Very Low density lipoprotein (VLDL)	37.84 ± 12.33	32.89 ± 10.24	0.010 (S)

The mean Total cholesterol, Triglycerides, LDL, VLDL were higher and HDL was lower among IgG + IgA Cp antibodies positive individuals, compared to IgG + IgA Cp antibodies negative individuals. The association of IgG + IgA Cp antibodies with Total cholesterol, Triglycerides, HDL, LDL and VLDL was found to be significant with a statistically significant P value of (0.055,0.006,0.000,0.008,0.010).

The association between IgG, IgA, and IgG+IgA Cp antibodies with various clinical diagnosis were studied. IgG Cp antibody positivity was seen more among Myocardial infarction patients(74%) and IgA Cp antibody positivity seen more among Unstable angina patients(78.9%) respectively.

Discussion

In today‟s world where most deaths are attributed to non-communicable chronic diseases, more than half of these are a result of CAD, Coronary artery atherosclerosis is considered as the main cause of CAD.

The known risk factors of CAD are not sufficient to explain all the epidemiological variables and fluctuations of the disease. These observations fuel renewed interest in a link between CAD and infectious agents. Strong clinical correlation between *Chlamydia pneumoniae* infection and CAD have been reported. Reinfection with *Chlamydia pneumoniae* is common and most acute infection in adulthood are reinfections. Chronicity is a feature of the genus and there is evidence that infection with *Chlamydia pneumoniae* may persist for many months. *Chlamydia pneumoniae* serology consists of various markers that help in detecting a previous or an ongoing infection. The possession of IgG antibody to *Chlamydia pneumoniae* denotes either previous exposure (with antibody persistence) or chronic or latent infection. 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, while patients having both IgG and IgA antibody may reveal chronicity or persistent active infection. [1] In the present study, an attempt was made to know the association and significance of *Chlamydia pneumoniae* infection in CAD by measuring IgA and IgG antibody in angiographically proven CAD patients.

Table 8: Comparison of age group of CAD patients with various studies.

Study Series	Year	Age Group (50-59.5 Years)
Lisa A. Jackson et al [10]	2000	49%
El-Sayed M.Ezzat et al [11]	2006	35.1%
Lt Col A Agarwal et al [14]	2007	71.05%
Mehvash Haider et al	2011	36.50%
Present study	2013	41.1%

In the present study, majority of CAD patients was seen in the age group between 50-59.5(41.1%), which is comparable with other studies.

Table 9: Comparison of Smokers among CAD patients with other studies

Study Series	Year	Smokers (%)
Hahn. DL et al [13]	1992	30.2%
Hammed A. Ali et al [12]	2010	67.1%
Mehvash Haider et al	2011	30.15%
Present study	2013	46.7%

In the present study, 46.7% of CAD patients were smokers which are comparable with other studies.

Table 10: Comparison of Diabetics among CAD patients with other studies

Study Series	Year	Diabetics (%)
Maryam Ghasemi et al [15]	2008	32.2%
Hameed A. Ali et al [12]	2010	74.2%
Present Study	2013	41.1%

In the present study, 41.1% of CAD patients were diabetics which is comparable with the study of Maryam Ghasemi et al.

In the present study, 58.9% of CAD patients were *Chlamydia pneumoniae* IgA antibody positive, compared to controls (11%) which are statistically significant. A Statistically significant odds ratio of 4.9 was found. It is comparable with other studies. Vergassola. R et al 1997, showed high level of Cp IgG MIF in 82.3% of acute MI patients and 29.4% of Healthy controls (P=0.0000065), IgA MIF were 70.5% in Acute MI patients and 29.4% in Healthy controls (P=0.0042). High level of Cp IgG and IgA anti Lps were found, with a high prevalence rate of 76.4% and 64.7% in acute MI patients. Naoyuki Miyashita A et al 1998, they investigated the frequency of *Chlamydia pneumoniae* seropositivity in patients with Acute MI. The odds ratio were 2.2 for IgG and 2.7 for IgA .The mean titers of IgG and IgA were significantly higher in patients with acute MI than controls. Shimada K et al 1999, showed that prevalence of either IgA or IgG Cp was significantly higher (59% Vs 30%) P=0.04) compared with the control group. The odds ratio for CAD were 3.4 for the prevalence of IgA, 2.3 for the prevalence of IgG and 2.3 for the prevalence of IgA + IgG.

Leowattana et al in 2000, investigated the relationship between the presence of IgG, IgA of Cp 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. Turkoqlu. C et al in 2004, found a significant association between seropositivity to *C.pneumoniae* IgG and dyslipidemia and CRP in acute CAD, chronic CAD patients ($P<0.05$). M.Karimi et al in 2006, found that the mean antibody level against Cp in MI, chronic stable angina subjects and control subjects were 34.7, 11.4 and 3.6μ/ml respectively. Obtained difference was found to be statistically significant ($p<0.001$). Yavuz MT et al 2006, showed the IgA and IgG Cp seropositivity were significantly associated with the presence of CAD ($P=0.005$) and were independent predictive factors for the severity of coronary atherosclerosis ($P=0.005$). S.Mazzoli et al in 1998, showed the high prevalence rate and high titers of IgG and IgA specific anti *C.pneumoniae* antibodies in Acute MI Population. The study by Puneet Goyal et al in 2006 showed that *C.pneumoniae* seropositivity was significantly associated with atherosclerotic CAD. Combined seropositivity to *C.pneumoniae* and *Mycoplasma pneumoniae* was higher in CAD patients with MI as compared to CAD patients without MI. [16]

Conclusion

Coronary artery disease is the leading cause of death and disability in the world. The main pathological process leading to CAD is atherosclerosis (the accumulation of fatty materials leading to arterial wall thickening). Several important risk factor for atherosclerosis like hyperlipidemia, hypertension, diabetes mellitus, smoking, family history have been discovered, but much of the risks remains unexplained. Current evidence indicates that inflammatory process is implicated in the initiation and the evolution of atherosclerotic process.

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