Chronic infections & coronary artery disease with special reference to *Chalmydia pneumoniae*

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Background & objectives: Studies on cardiovascular diseases (CVD) in India have shown about 10-20 per cent of cases with no obvious risk factors, raising a suspicion of infections as a cause. There is a paucity of data on this possible role of infections. This study was, therefore, undertaken to find out the association between infection due to *Chlamydia pneumoniae* and other organisms and coronary artery disease (CAD).

Methods: Patients with CAD were selected in group I (acute myocardial infarction, AMI) and group III (patients undergoing coronary artery bypass graft (CABG) surgery), and normal controls in group II. Routine biochemical, haematological and inflammatory tests [C-reactive protein (CRP), total leucocyte count (TLC), fibrinogen, ESR], serodiagnostic tests for IgA and IgG antibodies to *C. pneumoniae*, *Helicobacter pylori*, cytomegalovirus (CMV), *Mycoplasma pneumoniae* and Parvovirus B-19 by ELISA kits, *C. pneumoniae* antigen by microimmunofluorescence and PCR from endothelial tissue obtained at CABG were carried out. Aortic punch biopsies were done in patients who underwent CABG.

Results: Acute MI patients had a significantly higher association with accepted cardiac risk factors, lipid profile, inflammatory and thrombogenic tests. IgG and IgA antibodies levels against *C. pneumoniae* were not significantly different in the controls as against the AMI group. However, *C. pneumoniae* antigen seropositive group had significant association with HDL cholesterol, lipid tetrad index ($P<0.001$) and with triglycerides. Parvovirus B antigen was detected in 8.3 per cent of tissue specimens by PCR and of 44 patients with AMI (6.8%) were also positive for parvovirus B-19 IgG antibodies.

Interpretation & conclusions: There was no direct evidence of the involvement of *C. pneumoniae* and other infective agents and viruses in CAD. It is possible that such infections produce an indirect adverse effect on the lipid profile.

Key words *Chlamydia pneumoniae* - chronic infections - coronary artery disease

Worldwide, WHO has estimated in 2005 that out of 17 million deaths, 2/3 occurs in low and medium income countries including India[1,2]. Deaths in India are due to heart disease in 29 per cent and there has been a 7-fold increase in 4 decades (1960-2000) of the prevalence of IHD in epidemiological studies[3].

Risk factors have been the subject of many studies, dominated by hypertension, diabetes and smoking with multiple risk factors being in the majority[3]. However, in 10-20 per cent of cases, there is no obvious risk factor apart from the traditional and newer risk factors[3].

Infection has been suspected as a probable cause because of raised total leucocyte count (TLC), C-reactive protein (CRP), ESR and fibrinogen levels, in the acute coronary syndrome[4]. There have been several studies in industrialized countries[5-9] linking infection with coronary artery disease (CAD). Ieren *et al*[10] have shown the results for and against the assumption of infection. There have been four studies conducted in India in the recent past in this area[11-14].

We carried out this study to determine the relationship, if any of infections with suspected infective agents *e.g. Chlamydia pneumoniae,* cytomegalovirus (CMV), *Helicobactor pylori*, *Mycoplasma pneumoniae* and parvovirus B-19 with coronary artery disease (CAD) among Indians.

Material & Methods

This study was carried out at National Heart Insitute (NHI) of the All India Heart Foundation (AIHF), New Delhi, between 2000-2004. The Scientific Advisory Research & Ethics Committee of the All India Heart Foundation/National Heart Institute (NHI), New Delhi cleared the proposal. Written consent was taken from all the patients.

Selection of subjects: Consecutive patients with CAD attending outpatient department of NHI during the study period were selected for the study.

(1) Patients of unequivocal acute myocardial infarction (AMI) (Group I, N=200). These were the most likely subjects to show infection, if any.

(2) Normal controls (Group II, N=200). These were age matched controls selected from the Executive Check-up Wellness Programme of the NHI.

(3) Patients subjected to coronary artery bypass graft (CABG) (Group III, N=168)). These were proven cases of CAD (Table I). At the time of surgery, aortic tissue was obtained and evidence of infection was looked for. Only 168 subjects could be included in this group though originally planned to include 200. Aorta punch biopsies could be obtained only in 70 patients.

A special proforma was designed to record the clinical history of all subjects. Essential biochemical tests [glucose, lipid profile, kidney & liver function and inflammatory tests (total leucocyte count, TLC, C-reactive protein, CRP and fibrinogen)] were performed in the biochemistry laboratory of the National Heart Institute.

Serological evidence of chronic infection (IgG, IgA) was obtained by using standard test kits available commercially. ELISA(Savyon Diagnostic, Israel; Vircell SL., Spain; Pathozme, UK; Vir-Immun-Labor Diagnostika, Germany; Virion/Serion ELISA Classic, Germany; RIDASCREEN-Biopharm, Germany; Eurogenetics, Belgium), microimmunofluorescence (Vircell SL., Spain), direct immunofluorescence (Dako, Imagen, Denmark), latex agglutination (Ranbaxy, India)]. *C . pneumonia* antigen was also determined by fluorescent antibody test in 18 of 70 aortic biopsies tested. Eight of the 18 tissue biopsies were tested for antigen by DFA and because of negative results by PCR. PCR and DFA were carried out at the Chalmydia Reference Centre, All India Institute of Medical Sciences (AIIMS), New Delhi and immunoflorescence at the NHI, New Delhi.

The aortic endothelial tissues were collected in phosphate buffered saline (PBS) for immunofluorescence from patients undergoing CABG surgery and stored at – 70º C till further testing[11]. The slide was mounted with the mounting fluid and viewed under the fluorescent microscope (Nikon Corp., Japan) using 490 nm filters for the detection of the *C. pneumoniae* antigen. The positive and negative controls supplied with the kit were stained simultaneously. Multiple polymerase chain reaction (PCR) studies were also carried out on endothelial biopsies for *C. pneumoniae*, CMV, Enteroviruses and Parvo B-19.

Table I. Age/sex distribution of patients and control groups								
Age (yr)	AMI group Group I			Control group Group II			CABG group Group III		
	M	F	Total	M	F	Total	M	F	Total
< 40	32	Nil	32	53	4	57	Nil	Nil	Nil
40 - 50	90	3	93	64	27	91	21	1	22
51 - 60	1	Nil	1	Nil	Nil	Nil	60	15	75
Total	179	11	200	161	39	200	135	24	159

Statistical analysis: Univariate analysis was carried out to estimate the odds ratio and comparison between AML patients and controls using SPSS Software System . T test was used for comparison ? between the groups. Multiple logistic regressions by unconditional – conditional method was done to compare titres of *C. pneumoniae* antibodies in patients and controls at 95 per cent confidence interval.

Results

Maximum number of patients and controls were in the 40-50 yr age group. Male predominance was noted in all three groups (Table I).

Comparison of the lipid profile in AMI patients (group I) and controls (group II) showed a significant difference (*P*<0.001) in HDL cholesterol (< 35 mg/dl), and Lipid Tetrad Index, triglycerides level (*P*<0.01) and lipoprotein (a) (*P*< 0.05) (Table II) .

Among the known cardiac risk factors triglycerides were found to be significantly (*P*<0.001) higher in *C. pneumoniae* seropositive compared to seronegatives. Low LDL and higher levels of HDL and total cholesterol levels were also seen (*P*<0.05) in *C. pneumoniae* seropositive group compared to seronegative group (Table III).

There was no significant difference between the patients and controls with regard to IgG and IgA antibodies with any of the infective agents. Only 6.8 per cent AMI patients showed Parvovirus B antibodies (IgG), no kits were available for IgA (Table IV).

A follow up of eight patients where repeated samples were tested after 6 months to 2 years, no change in *C. pneumoniae* IgG, IgA antibody titres was observed. These antibodies persisted for a long time perhaps due to the presence of the *C. pneumoniae* in the tissues leading to chronic active infection.

C. pneumoniae antigen was also detected by direct fluorescent antibody (DFA) test in 18 of 70 (25.7%) aortic tissue biopsies tested (Table V). However, no association was seen between antigen detection and seropositivity. Eight of the 18 tissue biopsies positive for antigen by DFA gave negative results by PCR. By PCR, Parvovirus B was detected in 8 of 98 (8.1%) aortic tissues tested.

Discussion

In the present study, no significant difference in *C. pneumoniae* IgA and IgG levels was seen in the two groups (AMI and control). Similar results have been reported earlier[5,6]. Altman *et al*[6] studied 159 patients with arterial atherosclerotic disease and compared the result with 203 patients with a heart valve prostheses

Table II. Risk factors in AMI patients and controls						
Risk factors		AMI		Controls		
	Cutoff Value	No.	Mean ± SD	No.	Mean (SD)	*P* value
Age (yr)		199	46.8 ± 7.9*	200	45.0 ± 8.5	<0.05 SS
Hb	gm% (11.50-15.00)	194	13.9 ± 1.6	200	14.1 ± 1.7	>0.05 NS
ESR	mm/1st hr (0-42) wintrobe	194	16.1 ± 13.1	199	17.0 ± 12.8	>0.05 NS
TLC	/cmm (4-10)	199	10795.2 ± 3224.4***	200	7931.7 ± 1264.4	<0.001 SS
Glucose F	mg/dl (70-100)	186	122.9 ± 59.7***	200	95.2 ± 33.4	<0.001 SS
Urea	mg/dl (17-43)	199	30.2 ± 14.9*	193	26.6 ± 7	<0.05 SS
Cholesterol	mg/dl (< 200)	199	202.5 ± 41.96	200	209.5 ± 35.93	>0.05 NS
Triglycerides	mg/dl (< 150)	199	170.7 ± 3.05**	200	146.5 ± 71.09	<0.01 SS
LDL-Cholesterol	mg/dl(<100)	198	126.9 ± 35.6*	195	133.9 ± 31.0	<0.05 SS
HDL–Cholesterol	mg/dl(40-60)	198	41.16 ± 10.98***	196	46.4 ± 11.03	<0.001 SS
Chol:HDL	mg/dl M(3.8-5.9),F(3.1-4.6)	197	5.47 ± 3.5	193	5.93 ± 12.1	>0.05 NS
Lipoprotein(a)	mg/dl (14-30)	99	28.16 ± 24.96***	107	18.12 ± 20.04	<0.01 SS
Lipid Tetrad Index		99	21296.41 ± 23074.5***	98	10651.62 ± 15127.087	<0.001 SS
CRP	mg/L (< 6)	129	23.0 ± 34.4***	131	4.2 ± 7.5	<0.001 SS
Fibrinogen	g/L (1.5-2.77)	164	281.8 ± 164.2***	162	221.1 ± 98.9	<0.001 SS

Lipid tetrad index = Cholesterol x triglycerides x lipoprotein (a) / HDL; SS, statistically significant *P*< 0.05,< 0.001); NS, not significant *P* *<0.05; ** <0.01; ***<0.001 compared to controls.

and no demonstrable CAD. They concluded that *C. pneumoniae* infection was not an independent risk factor for CAD. In another prospective study, *C. pneumoniae* IgG seropositivity did not show any risk of future likelihood of myocardial infarction, and no association was found between *C. pneumoniae* infection and CAD[8].

Certain lipid risk factors (triglycerides, HDL and lipid tetrad index) were significantly higher in the *Chlamydia* seropositive group compared to the seronegative group. *Chlamydia* induces production of several cytokines leading to altered lipid metabolism, accumulation of serum triglycerides and a decrease in HDL[7-9]. Lipopolysaccharide, a bacterial component, binds in human serum to both HDL and LDL and makes LDL toxic to endothelial cells. Cytokines are potent inducers of neutrophils and free radical generation, which may facilitate oxidation of LDL, a key event in atherogenesis. Leukocyte activation may also lead to propagation of the atherosclerotic process[7,10]. Our study showed an association between *C. pneumoniae* antibodies and lipid risk factors.

It has been shown that despite a seronegative status, *C. pneumoniae* can be detected in atheromatous plaques by immune-cytochemistry, cell culture and polymerase chain reaction[8,10]. A significant association was found between *C. pneumoniae* seropositivity and an adverse lipid profile, indicating a possible indirect effect of infection with CAD.

Satpathy & associates from India[12] studied 62 end anteriotomy specimens of discarded coronary arteries of the CAD patients. Association between *C. pneumoniae* infection and CAD could not be established by genome or antigen detection. Goyal *et al*[13] investigated 30 CAD patients with no conventional risk factors scheduled for CABG and healthy blood donors. *C. pneumoniae* seropositivity was significantly, higher in CAD patients.

Jha *et al*[14,15] screened for *C. pneumoniae*, *H. pylori*, CMV and HSV-1 in different vascular locations of CAD patients using quantitative real-time (RT) PCR and suggested that *C. pneumoniae* may have caused

Table III. Comparison of some known cardiac risk factors with *C. pneumoniae* seropositivitly

Risk factors	No.	Seropositive No. (%)	Seronegative No. (%)
Cholesterol (> 200 mg/dl)	200	115 (57.4)*	91 (42.5)
Triglycerides (> 200 mg/dl)	87	56 (64.3)***	31 (35.6)
HDL (< 35 mg/dl)	85	49(57.6)*	36 (42.3)
LDL (>130 mg/dl)	187	105 (56.1)*	82 (43.8)
Chol: HDL (M<3.8, F < 3.1)	69	35 (50.7)	34 (49.2)
Lipoprotein a (>30 mg/dl)	59	32 (54.2)	27 (45.7)
Lipid Tetrad Index (>20,000)	54	23 (42.5)	31 (57.4)
Fibrinogen (> 400 mg/dl)	46	27 (58.6)*	`19 (41.3)
TLC (>11000 cumm)	78	43 (55.1)	35 (44.8)
CRP (Latex) (> 6 mg/l)	81	45 (55.5)	36 (44.4)
CRP (Elisa) (> 3 mg/l)	74	43 (58.1)	31 (41.8)

Lipid Tetrad Index = Cholesterol x Triglycerides x Lipoprotein a / HDL
P *<0.005 ****0.001 compared to seronegatives

Table IV. IgG/IgA antibodies in AMI patients and controls to some chronic infective agents

Infective agent	Anti-body	AMI		Control	
		No. positive/No. tested	%	No. positive/No. tested	%
Chlamydia pneumoniae	IgG	96/200	48.1	113/200	56.5
	IgA	77/200	38.5	71/200	35.5
Cytomegalovirus	IgG	118/141	83.6	159/200	79.5
Helicobacter pylori	IgG	69/110	62.7	62/108	57.4
	IgA	53/87	60.9	64/109	58.7
Mycoplasma pneumoniae	IgG	57/128	44.5	66/144	45.8
	IgA	23/88	26.1	17/88	19.3
Parvovirus B19	IgG	3/44	6.8	ND	ND
	IgA	No Kits available			

ND Not done

Table V. Association of *C. pneumoniae* antigen in aortic endothelial tissue biopsies and antibodies to *C. pneumoniae*

DFA test for *C. pneumoniae* antigen	No.	%	Antigen seropositive	
			No. positive / No. tested	%
Antigen positive	18	25.7	6/16	37.5
Antigen negative	52	74.3	32/48	66.6
Total	70	100		

Out of positives

chronic persistent infection in CAD. Our study in a small number of CAD patients also suggested continuing chronic infection.

In conclusion, no direct effect of infectious agents as causal for CAD could be shown. Our results showed an association between *C. pneumoniae* seropositivity and an adverse lipid profile. Further work needs to be done to confirm whether infectious agents have any causal effect in CAD.

Acknowledgment

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