Original Article

Serological Evidence of Chronic *Chlamydia pneumoniae* Infection in Coronary Artery Disease

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Abstract

Background : Recent studies have suggested that *Chlamydia pneumoniae* infection could be involved in atherosclerosis and related clinical manifestations such as coronary artery disease, carotid artery stenosis and myocardial infarction.
Methods : Serum IgG, IgM and IgA antibodies to chlamydia genus specific antigen were measured by enzyme linked immunosorbent assay (ELISA) in 100 cases of angiographically demonstrated coronary artery disease (CAD) and 100 randomly selected healthy individuals as controls after matching for age and sex. All the samples positive for chlamydia genus specific IgG antibodies were then subjected to *Chlamydia pneumoniae* species specific IgG antibody ELISA.
Results : Seroprevalence of chlamydia genus specific IgG antibodies in control group was 59% with an increase in seropositivity with increasing age. The overall seroprevalence of IgG antibodies was 76% in CAD group and the prevalence was significantly high in all age groups as compared to controls. The odds ratio was 2.20 for seropositivity of chlamydia genus specific IgG antibodies in patients with myocardial infarction (MI) and/or angina than in control group. No significant association was observed for IgA and IgM anti-chlamydial antibodies. The odds ratio for prevalence of *Chlamydia pneumoniae* species specific IgG antibodies in CAD patients increased to 2.55 in comparison to age and sex matched controls.
Conclusion : Current study supports the reported association between *C pneumoniae* infection and CAD in Indian population.

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Key Words : *Chlamydia pneumoniae*, Coronary artery disease

Introduction

Chlamydia pneumoniae, previously designated as the TWAR agent, was first described in 1986. It is an ubiquitous gram negative bacterium, an obligate intracellular parasite with a unique developmental cycle. It is a common respiratory pathogen in children and adults. It is involved in 5-10% of pneumonia cases in adults worldwide [1-3]. The expanding spectrum of *C pneumoniae* infection has been extended to atherosclerosis and related clinical manifestations such as coronary heart disease, carotid artery stenosis, aortic aneurysm, claudication (occlusion of the arteries of the lower extremities) and stroke [1]. Recent studies suggest that chronic *C pneumoniae* infection could be involved in chronic bronchitis and asthma [1,3]. These associations are determined by seroepidemiologic observations, case reports, isolation or direct detection of the organism in specimens, successful response to antichlamydial antibiotics or a combination of these methods [1]. Serological studies indicate a prevalence rate of 40-70% in many countries [4]. This study was undertaken to determine the seroprevalence of Chlamydial antibodies in Indian population and the association of chronic *C pneumoniae* infection with coronary artery disease.

Material and Methods

The study was designed as a case control study in a tertiary care hospital. Study population comprised 100 cases of coronary artery disease (CAD) who underwent coronary arteriography. Cases were defined as patients who had atleast one coronary artery lesion occupying atleast 50% of the luminal diameter on coronary arteriography (CART). 100 healthy individuals of both sexes (87 males and 13 females) selected randomly after matching for age and sex were taken as control. The mean age in CAD group and control group was 51.39 years and 51.15 years respectively.

Serum from blood samples of control and study group was stored at -20° C until analysis. All serum samples were first evaluated for *Chlamydia* genus specific IgM, IgG and IgA antibodies by commercially available enzyme linked immunosorbent assay (ELISA) kits (Novum Diagnostica, Germany). All the samples positive for *Chlamydia* genus specific IgG antibodies were then subjected to *Chlamydia pneumoniae* species specific IgG antibody ELISA (Savyon Diagnostics Ltd., Israel). Positive results were taken as indication of *C pneumoniae* infection.

The results obtained were analyzed statistically using chi square test.

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Results

The seroprevalence of Chlamydia genus specific IgG, IgA and IgM antibodies in the control group were 59%, 24% and 12% respectively (Table 1), with increase in seropositivity of IgG and IgA anti-chlamydial antibodies with increasing age.

Amongst the CAD group overall seroprevalence of chlamydia genus specific IgG antibodies was 76% in comparison to 59% in age and sex matched control group. The prevalence was found to be significantly high in all the age groups (Fig. 1). However, no significant association was observed for IgA and IgM chlamydial antibodies between CAD and control groups (Table 2). The odds ratio was 2.20 (95% confidence interval, 1.19 to 4.04; p value= 0.01) for seropositivity of *Chlamydia* genus specific IgG antibodies in patients with MI and/or angina than in control group.

Chlamydia pneumoniae species specific IgG antibody seropositivity was also found to be significantly high in all age groups in comparison to age and sex matched controls (Fig. 2). The overall seroprevalence of *Chlamydia pneumoniae* species specific IgG antibodies was 61% in CAD group as compared to 38% in control group (Table 3). The odds ratio increased to 2.55 (95% confidence interval, 1.44 to 4.51; p value=0.0011) for prevalence of *Chlamydia pneumoniae* species specific IgG antibodies in CAD patients as compared to age and sex matched controls.

Discussion

Since the discovery of Chlamydia in 1907 by Helberstaedter and Von Prowazek, as inclusion bodies in ocular scrapping, this organism has received an active consideration from the different workers all over the world. A wide variation of prevalence of antibodies to this infection is partly due to different types of tests used for antibody detection by different workers like microimmunofluorescence (MIF), complement fixation (CFT) and enzyme immunoassay (EIA), arbitrarily selected antibody titre cut offs and cross reactivity with others species.

The presence of anti-chlamydia IgG antibody is indicative of chlamydial infection at an undetermined time. IgG antibodies persist for long periods and decline very slowly. High levels of IgG antibodies are of diagnostic value in chronic/ systemic chlamydial infection provided there are no IgM antibodies [1]. In our study overall seroprevalence of *Chlamydia* genus specific IgG antibodies was 59% with the higher prevalence among males (60.91%) than in females (46.15%). The seropositivity increased with increasing age from 50%

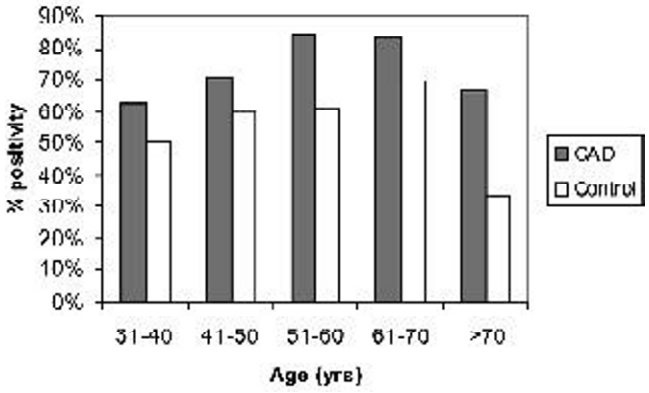

Fig. 1 : Seropositivity of *Chlamydia* genus specific IgG antibodies in CAD vs control group

Table 1

Seroprevalence of *Chlamydia* genus specific antibodies in control group

Age Group	IgG			IgM			IgA		
	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
31- 40 yrs	9	9	18	3	15	18	2	16	18
41- 50 yrs	18	12	30	4	26	30	5	25	30
51- 60 yrs	22	14	36	4	32	36	10	26	36
61- 70 yrs	9	4	13	-	13	13	5	8	13
>70 yrs	1	2	3	1	2	3	2	1	3
Total	59	41	100	12	88	100	24	76	100

Table 2

Seroprevalence of *Chlamydia* genus specific antibodies in CAD group

Age Group	IgG			IgM			IgA		
	Positive	Negative	Total	Positive	Negative	Total	Positive	Negative	Total
31- 40 yrs	10	6	16	1	15	16	3	13	16
41- 50 yrs	22	9	31	4	27	31	6	25	31
51- 60 yrs	32	6	38	8	30	38	5	33	38
61- 70 yrs	10	2	12	2	10	12	3	9	12
>70 yrs	2	1	3	-	3	3	-	3	3
Total	76	24	100	15	85	100	17	83	100

OR: 2.20; $?^2$: 6.587; p value : 0.0102

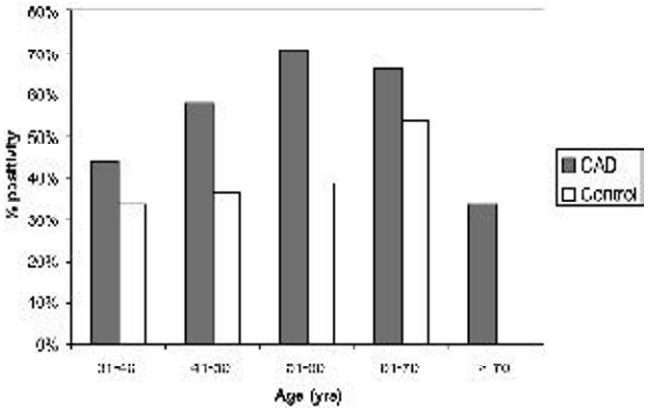

Fig. 2 : *Chlamydia pneumoniae* IgG seropositivity in CAD vs control group

Table 3

***Chlamydia pneumoniae* IgG seropositivity in CAD vs control group**

Age Group	Study Group (CAD) (n=100%)	Control Group (n=100%)
31-40	7 (43.75)	6 (33.33)
41-50	18 (58.06)	11 (36.66)
51-60	27 (71.05)	14 (38.88)
61-70	8 (66.66)	7 (53.84)
> 70	1 (33.33)	0 (0)
Total	61 (61)	38 (38)

OR: 2.55; $?^2$: 10.58; p value : 0.0011

at the age of 40 years to 69% at 70 years.

IgA antibodies have shown to be reliable immunological marker of primary, chronic and recurrent infections. These antibodies disappear rapidly and persistence of elevated IgA antibody titres is considered a sign of chronic infection [5]. However, Wang et al [6], have suggested in a follow up study of persons with long lasting IgG antibodies that a large portion of subjects never produce IgA antibodies for unknown reasons. Therefore, the role of IgA antibody as a marker for chronic *Chlamydia* infections is questionable. In this study we found low seroprevalence of IgA antibodies (24%) in general population as compared to IgG (59%). Seroprevalence of IgA antibodies also followed the rising trend with increasing age (12.5% in age group 31-40 years to 66.66% in >70 years).

Detection of IgM antibody is diagnostic of primary and/ or acute infection [1]. Overall seroprevalence of IgM in our control group was 12%. This marker has been used to differentiate acute from chronic *Chlamydia* infection.

Chlamydia pneumoniae accounts for majority of human chlamydial infections, though 90% of cases produce few or no symptoms [4]. Population prevalence of antibodies to *C pneumoniae* is approximately 30% in middle aged adults worldwide [7]. Kaftan et al [8], reported 34% seropositivity for IgG antibodies to *Chlamydia pneumoniae* in Japanese population while Kaykov et al [9], reported a seroprevalence of 33% in the Israeli population using ELISA.

In our study all the positive samples for *Chlamydia* genus specific IgG antibodies were subjected to *Chlamydia pneumoniae* species specific IgG antibody ELISA. If we consider that all the samples negative with *Chlamydia* genus specific IgG antibodies are also negative for species specific IgG antibodies, then the seroprevalence of *C pneumoniae* IgG antibodies in the control group was 38%.

Since no significant difference was found in proportion of patients positive for *Chlamydia* genus specific IgA and IgM antibodies in study and control group we saw no merit in evaluating all the samples for complete *Chlamydia pneumoniae* antibody profile.

Established risk factors account for no more than 30% of CAD cases, therefore search continues for other modifiable risk factors. In recent years there has been renewed interest in infectious theory of atherosclerosis. The link between *C pneumoniae* and atherosclerosis has been continuously strengthened by various studies including seroepidemiological, pathology based, animal models, cell biology and human antibiotic treatment trials [7,10,11]. First such serological study was done in Finland by Saikku et al [12], in 1988 by means of the microimmunofluorescence (MIF) test, when approximately two fold higher risk of IgG and/or IgA antibodies to *C pneumoniae* MOMP was reported in patients with myocardial infarction or angina than in similar control subjects. Subsequent serological results have been broadly confirmatory with odds ratios of 1.5 – 10 (average OR ~ 2.5) [13]. These studies were done in different populations, used different criteria for cases, adjusted for potential confounders to differing degrees, and were, therefore, prone to different biases. The general consistency of their findings in a total of 2700 cases and 5000 controls, supports the existence of serological association between *Chlamydia pneumonia* and CAD [13].

In the present case control study we found odds ratio of 2.20 (95% CI, 1.19 to 4.04; p value = 0.01) for seropositivity of *Chlamydia* genus specific IgG antibodies in patients with MI and/or angina than in control subjects. The odds ratio increased to 2.55 (95% CI, 1.44 to 4.51; p value=0.0011) for prevalence of *Chlamydia pneumoniae* species specific IgG antibodies in CAD patients as compared to age and sex matched controls.

The result of the current study supports the previously reported association between *C pneumoniae* infection.

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.

Further prospective, cohort studies are needed to investigate the association between *C pneumoniae* infection and the development of atherosclerotic disease. Additional laboratory studies are also needed to suggest mechanisms by which such an infection could contribute to atherogenesis.

Conflicts of Interest

None identified

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CORP NEWS

CME SESSIONS ON SELECTED PROFESSIONAL PAPERS

56TH ARMED FORCES MEDICAL CONFERENCE: 2008

1. It has been decided to organise six Continuing Medical Education (CME) sessions, during the above conference scheduled to be held at the Armed Forces Medical College, Pune in the month of February 2008 as per the details below:-
   (a) Medicine & allied Subjects.
   (b) Surgery & allied Subjects.
   (c) Pathology including Microbiology, Transfusion Medicine, Forensic Medicine and Biochemistry.
   (d) Preventive and Social Medicine.
   (e) Hospital Administration, Anatomy, Physiology and Pharmacology.
   (f) Dental Surgery.
2. The criteria for selection of papers for CMEs in general, will be in terms of originality, scientific content and methodology adopted. Rejection of a paper should not be regarded as a reflection of its merit, since diversity and balance are also considered in the selection of papers for presentation.
3. Wide publicity may please be given to this subject matter and medical, dental and MNS officers be encouraged to prepare papers of high quality which are worthy of presentation in the above CME programmes.
4. Papers for presentation should be submitted in an article format as per the guidelines to authors printed in Jan 2007 issue of Medical Journal Armed Forces India. The first page of paper must bear a heading **Paper for CME** (indicating the subject). Papers received in conformity with the correct format will only be taken up for consideration. **Seven copies** of each CME paper are to be submitted through proper channel so as to reach this office latest by **31 Aug 2007.**