

CHLAMYDIA PNEUMONIC INFECTION IN CORONARY HEART DISEASE IN BASRAH

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ABSTRACT

Aim: The aim of the present study was to determine the relationship between *Chlamydia pneumoniae* infection and coronary artery disease with classical risk factors in Basrah patients.

Background: Chronic inflammatory diseases might play significant role in the pathogenesis of atherosclerosis. We studied the differences between the percentage of immunoglobulin G against *Chlamydia pneumoniae* among Coronary Heart Disease patients and control subject.

Method: Antibody titer against *Chlamydia pneumoniae* by microimmunofluorescence method and C-reactive protein by agglutination method was determined among 225 patients proven Coronary Heart Disease and 180 control subject. Lipid risk and non-lipid risk factors in Coronary Heart Diseases patients was determined.

Results: *Chlamydia pneumoniae* seropositivity 1/64 titer was significantly higher in patients than control group (46.6% versus 25.5%) respectively; $P < 0.01$; odd ratio 2.64; CI (1.62; 3.46). Positive C-reactive protein ($\geq 6\text{mg/l}$) was significantly higher in patients (53.3 versus 39.4; $P < 0.001$; odd ratio 2.21; CI (1.46- 3.11)), than control group. All percentages of lipid and non-lipid risk factors in patients were significantly higher than control group.

Conclusion: We have demonstrated a significant rise in *Chlamydia pneumoniae*-specific immunoglobulin G and C-reactive protein levels is associated with Coronary Heart Disease.

INTRODUCTION

The search for new risk factors that can satisfactorily explain the prevalence and severity of coronary heart disease has brought into focus the role of infections, particularly the association of *C. pneumoniae* with CHD.^[1,2] Although many studies are available from western countries, the present study is undertaken for the first time in Iraq, remains a major cause of human mortality and morbidity worldwide. Classical risk factors for atherosclerosis fail to account fully for the wide variations in CHD prevalence and/or severity between different populations. The primary lesion is the atherosclerotic plaque in the intima of particular arteries, and the histological process is an inflammatory to injury. Hallmarks of chronic inflammation are present within both early (*fatty streak*) and advanced lesions with plaque rupture.^[3] The possibility that infectious agents may trigger a cascade of reactions leading to inflammation, atherogenesis, and vascular thrombotic events has recently been appraised.^[4] Clinical studies have associated chronic infection; due particularly to *Chlamydia pneumoniae* with atherosclerotic lesions and clinical coronary heart disease.^[5,6] A number of studies have indicated that seropositivity to *C. pneumoniae* with elevated concentrations of C-reactive protein (CRP) titers have a role in progressing CHD.^[1,7] To test the hypothesis that chronic *C. pneumoniae* infection is a cause of

CHD, the presence of specific IgG has often been taken to be a marker of chronic infection. Numerous such cross sectional^[8, 9] studies have been reported and their results have been extensively reviewed. The present study was designed to verify the theory suggesting that infection, particularly *Chlamydia pneumoniae*, is associated with CHD and to investigate the association between *C. pneumoniae* infections with the classical CHD risk factors in Iraqi persons.

MATERIALS AND METHODS

A case-control study was performed during the years 2007-2008. Cases were 225 patients admitted to Al-Sadder Teaching Hospital/Basrah/Iraq with CHD and normal persons were as control group; both groups were sex and age matched. Their age ranged (30-75) years. Full history taken, physical examination and investigations requested were: Electrocardiogram (ECG), exercise test, fasting blood sugar, serum total cholesterol, serum low-density lipoprotein (LDL) and serum triglycerides. Serologic screening for serum CRP and anti-chlamydial antibodies underwent a standard evaluation. In the present study 1/64 IgG titers was considered as a seropositive antichlamydial antibodies and ($\geq 6\text{mg/l}$) of CRP as cut-off was serum seropositive as mentioned by manufacturer. Antibody titers to *C.*

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pneumoniae were measured by a microimmunofluorescence (MIF) method, using *C. pneumoniae* CM-1 (ATCC VR-1360) strain as antigen (Vircell, S.L, Granada, Spain).^[10] The CRP was determined by agglutination method using CRP-latex kit (Ref.40111, Chemolax A Spain).^[11] Any patient with respiratory infection or urogenital infection was rolled out in this study. Statistical analysis was performed by using SPSS version (11) included univariant analysis. Comparisons between percentages were done using chi-squared test. A p-value <0.05 was considered statistically significant.

RESULTS

Prevalence of selected risk factors among patients and controls is shown in Table-1. Statistically significant associations were found between age, smoking, hypertension, and family history of CHD, diabetes mellitus, hypercholesterolemia, hypertriglyceridemia and hyper LDL and CHD. CRP seropositivity ($\geq 6\text{mg/l}$) was significantly higher in patients than control subjects (53.3 % versus 39.4%); $P < 0.001$ respectively; OR 2.21; CI (1.46- 3.11).

Table 1. Distribution of selected risk factors among patients and controls.

Risk factors	Patients(225) Positive (%)	Controls(180) Positive (%)	P-Value	OR (95%CI)
Age>50 years	175 (77.7)	125 (69.4)	0.05	1.42 (1.01;2.27)
Current smoking	151 (67.1)	62 (34.4)	0.001	2.04 (1.21;3.19)
Family history	121 (53.8)	31 (17.2)	0.001	2.367(1.78;3.32)
History hypertension	1175 (52)	50 (27.8)	0.05	1.95 (1.14;2.85)
Hypercholesterolemia	91 (40.4)	23 (12.8)	0.05	4.4 (1.14;2.85)
Hypertriglyceridemia	103 (45.7)	27 (15)	0.05	4.94 (3.86;5.97)
Hyper LDL	154 (68.4)	39 (21.6)	0.001	7.7 (5.58;8.35)
Diabetes mellitus	167 (74.2)	36 (20)	0.001	2.15 (1.25;3.78)
CRP $\geq 6\text{mg/l}$	120 (53.3)	71 (39.4)	0.001	2.21 (1.46;3.11)
<i>C. pneumoniae</i> IgG titer> 1/64	105 (46.6)	46 (25.5)	0.001	2.64 (1.62;3.46)

LDL-C: low- density lipoprotein-cholesterol. OR: odds ratio, CI: confidence interval.

C. pneumoniae seropositivity was significantly higher in patients than control group (46.6% versus 25.5%) respectively ($P < 0.01$; OR 2.64; CI 1.62; 3.46). The percentages of selected risk factors among patients with seropositive and seronegative of *C. pneumoniae* among patients are summarized in Table-2. The percentage of seropositive patients with diabetic, smoker and positive family history to CHD were significantly different than those patients who were seronegative of *C. pneumoniae* (53.2% VS

46.6%, 55% VS 45% and 62% VS 38%) respectively ($P < 0.05$). No significant difference was found in the percentage of hypertensive among seropositive (53.8%) and seronegative patients (46.2%). The result of univariate analysis showed that patients with seropositive IgG titer were (1.79) times more in diabetics, (1.09) times more likely to be smokers, and (0.97) times likely to have positive family history compared to seronegative patients.

Table 2. Percentages of selected Risk factors among seropositive and Seronegative to *C. pneumoniae* among CHD patients.

Risk factors	Seropositive seronegative Patients		Crude or	95% confidence interval
	No. (%)	No. (%)		
D.M	39 (53.2)*	34 (46.6)	1.79	1.01-3.14
HT	63 (53.8)	54 (46.2)	1.14	1.24-3.67
Smokers	83 (55)*	68 (45)	1.09	0.64-1.86
Positive FH	75 (62)*	46(38)	0.97	0.57-1.66

*P < 0.05; D.M: diabetes mellitus; HT: hyper tension; FH: family history, OR: odd ratio

(Table-3), shows seropositive patients (79.2%) who has positive CRP (≥ 6 mg/l) than those seronegative (20.8%) P< 0.001. A significant

differences were recorded between seropositive patients who has positive CRP (≥ 6 mg/l) (79.2%) and control group (54.9%) P<0.01.

Table 3. Correlation between antichlamydia IgG and CRP titers in study population.

	CHD patients		Control	
	CRP ≥ 6 mg/l No. (%)	CRP <6 mg/l No. (%)	CRP ≥ 6 mg/l No. (%)	CRP <6 mg/l No. (%)
<1/64	25 (20.8)	95 (90.5)	32 (45.1)	100 (91.7)
$\geq 1/64$	95 (79.2)**	10 (9.5)	39 (54.9)*	9 (8.3)
Total	120	105	71	109

X= 16.02; *P- value 0.01 (seropositive IgG patients with positive CRP vs. control subjects).

**P-value 0.001(seropositive IgG patients with positive CRP vs. seropositive IgG patients with negative CRP); CRP: C - reactive protein.

DISCUSSION

The majority of patients with coronary heart disease were >50 years as observed in our study and were in accordance with the previous findings.^[12, 13] Our findings are consistent with the results of Schunner,^[14] who found a significant difference between positive family history of CHD patients & control subjects (30% VS 18.5%). All other non-lipid and lipid risk factors in present study of CHD patients were significantly different than control subjects. These results are consistent with other studies.^[15-19] Among the different inflammatory markers analyzed so far, CRP seems to be the best marker of outcome in ischemic heart disease, partly because of the standardization of its measurement, low cost and wide availability, and partly because of its biological characteristics.^[20] The present study shows that CRP seropositivity (≥ 6 mg/I) was significantly associated with patients than in control subjects (53.3 V 39.4) respectively (P<0.01; OR 2.21; CI 1.46-3.11). However, Ridker, et al^[21] has considered CRP levels above 15mg/I indicative to clinically relevant inflammatory condition, which is in correspondence to our results, where high percentages of high levels of seropositive CRP were found in patients than in control

subjects. To verify whether the chronic infection theory could be the cause of coronary artery disease; the presence of specific IgG antibody of *C. pneumoniae* infection was adopted as to be a marker of chronic infection or of past exposure to infection. This study reveals that IgG seropositivity rate and levels against *C. pneumoniae* were significantly higher in patients (46.6%) with odds ratio 2.64 (95% CI- 1.62 to 3.46) than those of control subjects (25.5%). It is difficult to compare the results of most seroepidemiologic studies on the association between *C.pneumoniae* and CHD, because these were done on distinct populations, used various cut-off titers and antibody fractions for *C. pneumoniae* seropositivity.^[22] This study, defined *C.pneumoniae* seropositivity as an IgG titer of 1:64, better than those used less than this titer.^[22,23] 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.^[24,25] Many studies [6,1] indicated that seropositivity to *C. pneumoninae* with elevated concentrations of CRP titers is more frequent in CHD patients than in control subjects; and that risk has increased five times if elevated CRP was present.^[12] Our results in (Table-2,3) confirmed these hypothesis and in agreement with other studies.^[12, 26, 27]

In conclusion, a significant rise in *C. pneumoniae*-specific IgG and CRP levels is associated with acute ischemic heart disease.

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