

Seroepidemiologic Study of *Chlamydia pneumoniae* in Infarct Patients, Yazd, Iran

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Abstract: Myocardial Infarction (MI) is one of the leading causes of death in the world. Today, it is well known that some bacteria and viruses play a vital role in MI manifestation. *C. Pneumoniae* is considered to be one such bacterium. The general purpose of this cross-sectional study was to determine the correlation between MI and *C. Pneumoniae* infection. 103 infarcted patients along with 75 healthy people as control with similar demographic status were selected for this study. Initially, IgG and IgM titres were done using ELISA technique for detecting antibodies against *C. Pneumoniae* and then the serum was further examined for determination of sugar, CRP, cholesterol and triglyceride levels. Simultaneously, demographic features such as blood pressure, family history and addiction to smoking were recorded. Results showed that 92 (89.3%) of serum samples from patients and 58 (77.2%) of controls were positive for IgG, whereas only 2 patient's serum samples and none of the controls were positive for IgM. Relation of IgG levels with sex was statistically insignificant, but this relationship with age was significant ($p=0.03$). History of blood pressure in patients was recorded and had no significant relationship with IgG levels. Although the number of cases studied was limited, it can be concluded that *C. Pneumoniae* infection is prevalent in Yazd and this silent infection could be the causative factor of acute MI.

Key words: Myocardial Infarction • *C. Pneumoniae* • Seroepidemiology

INTRODUCTION

Atherosclerosis particularly myocardial infarction (M.I) is one of the most important causes of morbidity and mortality in the whole world. Smoking, diabetes, dyslipidemias and Hypertension are all well known as risk factors in heart diseases [1, 2].

Recently, several studies implicated that both viral and bacterial infections play a vital role in development of atherosclerosis [2, 4, 5]. Among different species reported, *C. Pneumoniae* has been high lighted the most in relation with coronary heart diseases [5, 6].

C. pneumoniae, an obligate intracellular bacterium, causes a variety of respiratory diseases [2]. A number of studies indicate that this pathogen is associated not only with respiratory diseases but also with chronic inflammatory diseases, such as atherosclerosis, endocarditis, asthma and arthritis [3, 7, 8].

Initially, evidence of this association was based on seroepidemiologic studies and more recently the presence of *C. pneumoniae* in atheromatous plaques has been shown by electron microscopy and immunocytochemical staining, as well as PCR testing of coronary carotid and aortic atheroma [7, 9, 10].

Several studies using PCR revealed that *C. Pneumoniae* has colonized the coronary artery during its infection in the body [9]. Recently, Ikejima and colleague [10]. Succeeded to detect *C. pneumoniae* from RBC of human's blood. This may be implicated that the bacterium has ability to parasite the blood cell and participate the heart related arteries through circulation [11].

The aim of this study was to evaluate the prevalence of *C. pneumoniae* infection among patients with MI, by antibody detection (IgM, IgG) using ELISA technique.

MATERIALS AND METHODS

The present study was a descriptive and cross-sectional in which 103 patients (75 male, 28 female) with acute myocardial infarction confirmed by cardiologist immediately after admission to emergency ward.

Consequently, 75 healthy individual (53 male and 22 female) were invited to participate the survey as control group. The control group had no any history of cardiovascular events and their gender together with age were tried to be matched as with cases.

A questionnaire was completed for each subject including patient's family history, symptoms and risk factors concerning heart disease such as: smoking, diabetes, hypertension and dyslipidemias. For each individual, blood sample was collected on admission in two tubes. Following centrifugation to separate the serum, one sample was kept at 4°C and the other samples was sent to the laboratory for further blood tests such as cholesterol, triglyceride, CRP, sugar, uric acid and urea.

Detection of antibody as IgG and IgM against *C. Pneumoniae* was performed according to manufacturer protocol (Helsinki, Finland). Following the method recommended by kit manual, the calculation and statistical analysis were done according to the given protocol.

Data were analyzed using spss program and then all patients and control groups with risk factors were eliminated from this survey.

The proposal of the present work was reviewed and considered by ethical committee and confirmed for further performance.

RESULTS

Table I summarizes the baseline characteristic of both case and control subjects of 103 admitted patients. 92 (89.3%) of cases and 58 (77.3%) of controls had a high titer of IgG to *C. Pneumoniae*. Using chi-square, the percentage of cases IgG compared to control was found to be significant (p 0.03). Measurement of odd ratio showed that risk of MI in IgG positive subjects were 2.45 greater than the IgG negative subjects. When the data were analyzed according to sex among male and female, the prevalence of IgG positive was found no significant in both case and control groups (p= 0.422). Note that IgM was negative in both, cases and control.

DISCUSSION

Traditionally, the cause of atherosclerosis has emphasized on classic risk profiles, including

Table 1: Prevalence of IgG against *C. Pneumoniae* in cases and control

Groups	* IgG +		IgG -		Total	
	No.	%	No.	%	No.	%
Cases	92	89.3	11	10.7	103	100
Controls	58	77.3	17	22.7	75	100
Total	150	84.3	28	15.7	178	100

* IgG detection in cases was significantly higher than the control group: p=0.03

Tables 2: Prevalence of IgG positive in both case and control according to sex

Group	Male			Female		
	IgG + (%)	IgG - (%)	Total	IgG + (%)	IgG - (%)	Total
Studied						
Case	66 (88)	9 (12)	75	27 (92.6)	2 (7.4)	29
Control	40 (75.5)	13 (24.5)	53	18 (18.8)	2 (18.2)	22

Correlation was non-significant using Chi-square (P=0.42)

hyperlipidemia, hypertension, diabetes, tobacco abuse, age, sex and familiar history of premature vascular disease [2].

In recent years, medical literatures have shown that *C. pneumoniae* plays a role in coronary atherosclerotic disease. This theory was highly supported when chlamydial related genes were detected using the most sensitive PCR technique [1, 2, 4].

The present study examined the sero status for *C. pneumoniae* in patients with an acute cardiovascular event. As Table 1 show a total of 103 serum samples were tested. 92 (89.3%) of patients and 38 (77.3%) of control groups were positive for IgG using ELISA technique. The results show that there is a correlation between MI and *C. pneumoniae* infection. In contrast to our finding Nobel *et al.* [12, 13] did not observe any correlation between the *C. pneumoniae* and MI. However, several seroepidemiology studies revealed from USA and some European countries have strongly supported the direct role of chlamydial infection in manifestation of coronary heart disease [4, 9, 14]. The difference between the results obtained from Nobel's and some other observers including a survey in Iran [12] could be related to low number of cases and control (total 116 in Nobel study) they applied in their investigation.

It also should be noted that only ELISA was used for detection of *C. pneumoniae* in the present study, whereas others recent study applied both ELISA and Micro immunofluorescence assay (IMA) in order to confirm their findings [5, 8, 9, 10]. However the results generated from our study would match the data collected from the past study.

Table 3: Prevalence of IgG against *C.pneumoniae* in both case and control groups

Group	Age	IgG +		IgG -		Total
		No.	%	No.	%	
Case N=85	30-44	13	86.7	2	13.3	15
	45-59	18	81.8	4	18.2	22
	60-	45	93.8	3	6.3	48
Control N=74	30-44	27	64.2	12	30.8	39
	45-59	19	74.2	5	20.8	24
	60-	11	100	0	0	11

Table 4: Correlation between IgG positive and blood pressure among case group

Cases n=103	Blood pressure	IgG + (%)	IgG- (%)	Total
	Yes	30(90.9)	3(9.1)	33(100)
No	62(88.6)	8(11.4)	70(100)	

Correlation between the IgG + and blood pressure was not significant using chi - square. (p=0.56)

As Table 2 indicates, 88% of infarcted men and 92.6 women were positive for IgG (p=0.42). Although the differences found to be non significant (p=0.42), but *Chlamydia* infection among women was found to be higher than those in men. This result is controverted with Grayston *et al.* [15] who revealed that men have higher risk in acquisition of *Chlamydial* respiratory diseases. This is probably because the men are generally more vulnerable to respiratory infection.

Aging is another risk factor for infarction [2]. As Table 3 reveals, the more IgG positive samples were seen among patients with 60 and over years old. Since we tried to minimize all the possible risk factors among the cases, therefore, it may be concluded that infarction of the patients was due to past *Chlamydial* infection and antibody complexes participated in heart's related arteries through passing time.

In order to eliminate the co-factors related to infarction manifestation, the history of blood pressure among cases were asked and recorded. As Table 4 shows, only 20 (14.42%) cases with high blood pressure were IgG positive, whereas 72 (64.40%) cases had no blood pressure. Since high blood pressure has a direct role in heart related diseases [2], therefore the results obtained from this study support our idea in that *Chlamydial* past infection was the main causative factor for heart problem among cases presented in this study.

Although, the case numbers were limited, but it may be concluded that the infection is prevalent among the residents in Yazd and the silent infection could be the causative factor in manifestation of acute M I.

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REFERENCES

- Danesh, I., P. whincup and M. Walker, 2000. *C. pneumoniae* IgG titers and coronary heart diseases: prospective study and Meta - analysis. *BMJ*. 321: 208-213.
- Kuvin, J.T. and C.D. kimmelstiel, 1999. Infectious cause of atherosclerosis. *Am. Heart J.*, 137: 216-26.
- Bonnet, F., 2000. Morlat p. Delevaux I and Gavinet AM, *et al.* A Possible association between *C. psittaci* infection and temporal arteritis. *Joint Bone Spine*, 67: 550-52.
- Moazed, T.C., C.C. Kuo, I.T. Grayston, L.A. Campbell, *et al.* 1996. *C. pneumoniae* infection. *AMJ Pathol.*, 148: 667-676.
- Tomioka, H., R. Fujiyama, H. Ohnishi, K. Tada *et al.* 2000. A case presenting sever respiratory failure with high antibody titers to *C. pneumoniae*. *Eur. Respir. J.*, 16: 108-111.
- Sessa, R., M. Dipleto, I. Santino, M. Piano *et al.*, 1999. *C. pneumoniae* infection and atherosclerotic coronary disease, *Am. Heart. I.*, 137: 1116-90.
- Saikku, P., M. Leionen, K. Mattila, M.R. Ekman *et al.* 1988. Serological evidence of an association of a novel Chlamydia, TWAR, with coronary heart disease and acute myocardial infarction. *Lancet.*, ii: 983-86.
- Wald, N.I., M.R. Law, J.K. Morris, Zhoux *et al.*, 2000. *C. pneumoniae* infection and mortality from ischemic heart disease. *BMJ.*, 2000.
- Grayston, J.T., C.C. Kuo, A.S. Coulson, L.A. Campbell, 1995. *C. pneumoniae* in atherosclerosis of the carotid artery. *Circulation.*, 92: 3397-3400.
- Ikejima, H., H. Friedman, G.F. Leparc *et al.*, 2005. Depletion of a resident *C. pneumoniae* through leukoreduction by filtration of blood for transfusion. *J. Clin. Microbiol.*, 43: 4580-4584.
- Mims, C., H.M. Dokrell, R.V. Goering *et al.*, 2004. *Medical microbiology*. Mosby Co., pp: 201-237.
- Frieman, M.G., A. Gatil, S. Greenberg and S. Kahane, 1999. Seroprevalence of IgG to the Chlamydia - like microorganism by ELISA. *Epidemiol Infect.*, 122: 117-123.

13. Kue, C.C., A. Shor, L.A. Campbell, H. Fukushi *et al.*, 1993. Demonstration of *C. pneumoniae* in atherosclerotic lesions of coronary arteries. J. Infect. Dis., 167: 841-840.
14. Noble, M., A. De-Torrente, O. Peter and D. Genne, 1999. No serological evidence of association between *C. pneumoniae* infection and coronary heart disease. Scand. J. Infect. Dis., 31: 261-264.
15. Weiss, S.M., P.M. Roblin, C.A. Gaydos, P. Cummings *et al.*, 1996. Failure to detect *C. pneumoniae* in coronary atheromas of patients undergoing atherectomy. J. Infect. Dis., 173: 957-996.
16. Eley, A. and M.B. Khalili, 1993. Epidemiology of *C. trachomatis* using nested PCR. Genitourin Med., 69: 239-241.
17. Grayston, J.T., 1986. Kuo CC wang sp and Altmany A new *C. psittaci* strain TWAR from acute respiratory tract infection, N. Eng. J. Med., 315: 161-168.