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## Correlation Between *Chlamydia pneumoniae* Infection and Lipid Profile in Patients with Cardiovascular Diseases

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### Abstract

Cardiovascular disease (CVD) is a class of diseases that involve the blood vessels or heart. *Chlamydia pneumoniae* has been considered as the most reasonable; also, it is able to increase and persevere inside vascular cells and to make the chronic inflammation in atherosclerosis. In this study, blood samples were subjected for molecular detection of *Chlamydia pneumoniae* by using conventional polymerase chain reaction (PCR) depending on *16S rRNA*. Seventy patients who suffer from cardiovascular diseases (angina, myocardial infarction and atherosclerosis) aged between 33-86 years have been investigated and compared to twenty of apparently healthy individuals were studied as a control group. Twenty-six samples (37.14) % revealed positive results for *Chlamydia pneumoniae* by PCR technique in blood samples of patients group, while all control samples were negative. No significant relationship was found among HDL, LDL, cholesterol, but the significant differences in the levels of triglyceride, VLDL between *Chlamydia pneumoniae* positive and negative within the patients' group were significant ( $P < 0.05$ ). The result of this study revealed that there was elevation of cholesterol and triglycerides level in patients their ages less than 50 years compared with other age groups although there was no significant relationship between HDL, VLDL, LDL and age; but generally certain patients with more than 60 years have the highest level of HDL, VLDL, LDL.

**Keywords:** *Chlamydia pneumoniae*, coronary artery disease, cardiovascular disease, lipid profile, PCR.

## العلاقة بين عدوى الكلاميديا الالتهابية وملف الدهون في المرضى الذين يعانون من أمراض القلب والأوعية الدموية

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### الخلاصة

صممت هذه الدراسة لتقييم العلاقة بين الدهون و *Chlamydia pneumoniae* في مرضى أمراض القلب والأوعية الدموية. خضعت عينات الدم جميعها للكشف الجزيئي لـ *C. pneumoniae* باستخدام تفاعل البلمرة المتسلسل (PCR) التقليدي اعتماداً على جين *16S rRNA*. تم فحص 70 مريض من الذين يعانون من أمراض القلب والأوعية الدموية (الذبحة الصدرية، تقلص عضلة القلب وتصلب الشرايين) الذين تتراوح أعمارهم

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بين 33-86 سنة، ومقارنة النتائج مع عشرين من الأفراد الأصحاء ظاهريا كمجموعة سيطرة. أظهرت النتائج ان ستة وعشرون عينة (37.14) % اعطت نتائج إيجابية لوجود *C. pneumoniae* بواسطة تقنية ال PCR في عينات الدم من مجموعة المرضى ، في حين كانت جميع عينات السيطرة سلبية. لم يتم العثور على علاقة ذات دلالة إحصائية بين HDL ، LDL، والكوليسترول ، ولكن الاختلافات في مستويات الدهون الثلاثية ، VLDL بين *C. pneumoniae* الإيجابية والسلبية ضمن مجموعة المرضى كانت كبيرة ( $P < 0.05$ ). اظهرت نتيجة هذه الدراسة أن هناك ارتفاعا في مستوى الكوليسترول والدهون الثلاثية لدى المرضى باعمار أقل من 50 سنة مقارنة مع الفئات العمرية الأخرى على الرغم من عدم وجود علاقة ذات دلالة إحصائية بين LDL ، HDL VLDL والعمر ؛ لكن بشكل عام وحتمى: المرضى الذين اعمارهم أكثر من 60 سنة كان لديهم أعلى مستوى من HDL VLDL و LDL .

## Introduction

Cardiovascular disease (CVD) is a class of diseases that involve the blood vessels or heart [1]. It includes coronary artery diseases (CAD) such as angina and myocardial infarction (commonly known as a heart attack). Other CVDs include peripheral artery disease like atherosclerosis, hypertensive heart disease, stroke, heart failure [2]. CVD arising from atherosclerosis will be the main cause of death in the worldwide [3]. Addition to the traditional cardiovascular risk factor that categories cover personal, biochemical, physiological characteristic. *C. pneumoniae* has been considered as the most reasonable, also, it is able to increase and persevere inside vascular cells and to make the chronic inflammation in atherosclerosis [4]. *C. pneumoniae* an intracellular, gram-negative bacterium is characterized by other bacteria by an exceptional life cycle. In this life cycle there are two functionally and morphologically separate cell types: the reproductive Reticulate Body (RB) and the infectious Elementary Body (EB) [5]. Atherosclerosis, a disease distinguished by the aggregation of fibrous elements and lipids in the large arteries [6]. *C. pneumoniae* contagion in monocytes influence the biophysical characterization of the membrane with changes in fluidity and lipid distribution [7] commonly recognized cytokines, especially IL-6, Tumor necrosis factor (TNF) and interleukin (IL)-1, which intermediate the host acute-phase reaction to inflammation and infection, also mediate variations in lipid metabolism. Chronic infection may influence the appearance higher of triglyceride and decrease HDL levels—both known risk factors for CHD [8]. In the presence of LDL, *C. pneumoniae* induces foam cell formation and simulates LDL oxidation, through chlamydial LPS and Hsp60, respectively. Scavenger receptors mediate oxidized LDL (oxLDL) uptake. Scavenger receptors mediate oxidized LDL (oxLDL) uptake [9]. For endothelial cells, the lectin-like oxLDL receptor (LOX-1) is the major receptor for uptake of oxLDL. *C. pneumoniae* has been shown to bind to the LOX-1 receptor, up-regulate LOX-1 expression, induce the expression of adhesion molecules and matrix metalloproteinases through LOX-1 activation, and promote uptake of ox-LDL. *C. pneumoniae* infection of macrophages inhibits the expression of the cholesterol transporters ABCA1 and ABCG1 which play critical roles in cholesterol efflux and homeostasis [10]. This study designed to evaluate the relationship between lipid profile and *Chlamydia pneumoniae* infection in cardiovascular diseases patients.

## Materials and methods

Seventy Iraqi patients with CVD (angina, myocardial infarction and atherosclerosis) ranged between 33-86 years old were involved in this study. They were collected from Ibn Al-Bitar specialist center for cardiac surgery in Baghdad between December 2017 to March 2018. All patients were non-hypertension non-diabetic, patients and none of them had a history of any underlying chronic infections or autoimmune disease. This study was carried out after procurement permission from the requisite ethics committee and informed consents from patients. Patients samples have been investigated and compared with twenty of apparently healthy volunteers were studied as control group with age ranged between 30-86 years. The DNA extraction from blood specimens was carried out according to the manual of manufacturer of (Gene aid Company, Korea) that used to detect the presence of 16S rRNA of *C. pneumoniae* using conventional PCR.

## Molecular detection of 16S rRNA gene of *C. Pneumoniae* by PCR technique

The DNA extraction from blood samples was achieved according to the manual of manufacturer of (Gene aid Company, Korea). High production of purified DNA is isolated the DNA integrity and quality were projected by re-forming the DNA bands by electrophoresis on agarose 1% for 45 min. The

bands seem sharp single not spread and have no smear which may result from DNA degradation It was used to detect the presence of *16SrRNA* of *C. pneumoniae* using conventional PCR. The molecular study achieved in Biotechnology Research Center, AL-Nahrain University department of medical biotechnology. Double of *16SrRNA* primers were used for amplification of certain gene for all samples. Each run mixture contained 1.5 $\mu$ l of forwarding primers, 1.5 $\mu$ l of reverse primer, 7 $\mu$ l of DNA, 5 $\mu$ l of master mix and 10 $\mu$ l of nuclease free water, to get a whole volume 25 $\mu$ l. In 1.5 % agarose gel, the PCR products were run as listed in the Table-1

**Table 1**-Primers sequences and PCR condition to detect *Chlamydia pneumoniae* genes

Gene		Primer sequence	Size of product	PCR condition	Reference
<i>C.pn</i> <i>16S rRNA</i>	Forward	TGACAACTGTAG AAATACAGC	463	95 $^{\circ}$ c 2.5min 1X	[11]
	Reverse	ATTTATAGGAGA GAGGCG		94 $^{\circ}$ c 1min 53 $^{\circ}$ c 20sec 30X 72 $^{\circ}$ c 40sec	
				72 $^{\circ}$ C 5min 1X	

For preparation of 2.0% agarose gel, 2.0 gm of pure agarose powder was dissolved to a final volume of 100 mL in 1X Tris Borate EDTA (TBE) buffer. The mixture was boiled in a microwave oven until the agarose was dissolved. The agarose gel allowed to cool to 45- 50 $^{\circ}$ C. Afterward, 0.2 $\mu$ L of 10 mg/mL Red Safety Stain was added and mixed thoroughly by gentle swirling. The molten agarose solution was poured into the casting chamber. Air bubbles were removed from the gel by poking them with a micropipette tip. A comb was set on the chamber and soaked in agarose gel; and left for 30 minutes at room temperature for solidification and then the comb was removed from the gel. The gel cast tray was put in the electrophoresis tank and filled with 1X TBE buffer sufficient to cover the entire gel. Subsequently, each well was loaded by adding a 8 $\mu$ L of PCR product (DNA sample) premixed with 2 $\mu$ L of the loading dye while 5 $\mu$ L of DNA ladder (A50-bp DNA ladder, GENETBIO, Korea) was used as a size marker. The DNA was electrophoresed at 80 Volts for 45 min. Finally, for DNA visualization the gel was examined and documented under UV light.

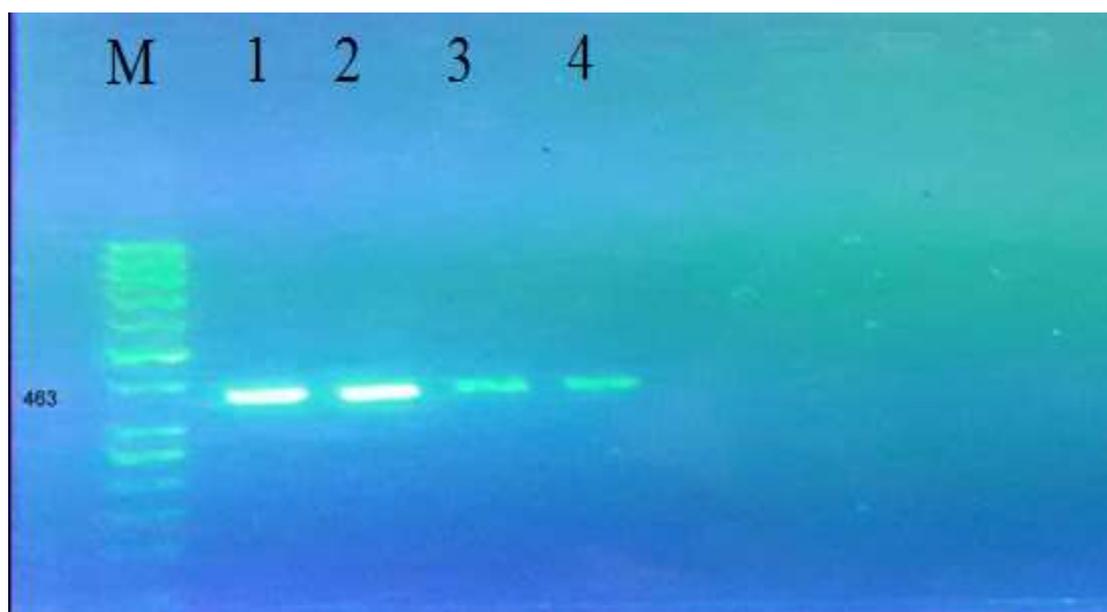
#### Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to show the effects of different factors in the study parameters. Chi-square test was used to significant compare between percentage and least significant difference –LSD, or T-Test was used to significant compare between mean values.

#### Result and Discussion

##### Molecular Characterization

The result of gel electrophoresis for DNA bands according to genes *16S rRNA* gene of *C. pneumoniae* in patients, revealed that twenty six (37.14% ) were positive *16S rRNA* in their blood samples with molecular length 460 base pairs, while all control sample was a negative (Figure-1 and Table-2). On the other hand, the patients with age below 50 years have the highest cholesterol levels as showed in Table-3 and the rate of male was 92.30 % ( 24/26); While female infection percentage was 7.69% (2/26) as illustrated in Table-4.



**Figure1**-Amplification PCR product *16S rRNA Chlamydia pneumoniae* on 2% agarose electrophoresed at 80 volt for 45 min and photographed under ultraviolet Trans illuminator. M: molecular marker (50 bp DNA ladder), stained with red stain bands in the gel.

**Table 2**-Distribution of the studied samples according to PCR results

Studied groups	No. (%)
<i>16S rRNA</i> positive (CVD patients)	26(37.14)
<i>16S rRNA</i> negative (CVD patients)	44(62.86)
<i>16S rRNA</i> negative (healthy control)	20(100%)
Chi-square ( $\chi^2$ )	9.073 **
** high significant differences (p<0.01).	

Lipid profile (cholesterol, triglyceride, HDL, LDL, and VLDL), as well as urea and creatinine, were done for all collected samples, All these parameters were within normal range for the healthy control group, so the comparison was made within the CVD patient group as shown in Tables-(3,4 and 5).

**Table 3**-Distribution of lipid profile according to age of CVD patients

Parameters	Age (year)			LSD value
	Less than 50	50-60	More than 60	
Cholesterol mg/dl	206.57±18.26	165.45±10.30	169.04± 10.32	40.42 *
Triglyceride mg/dl	320.71±19.51	305.25±23.84	311.54± 23.52	53.79 NS
HDL mg/dl	32.75 ± 1.54	35.85 ± 1.76	37.23 ± 2.07	4.88 NS
VLDL mg/dl	64.89 ± 4.04	62.00 ± 4.98	65.18 ± 4.32	10.81 NS
LDL mg/dl	95.23 ± 8.63	85.60 ± 8.24	101.53 ± 7.47	23.86 NS
Urea mg/dl	33.57 ± 2.04	35.04 ± 3.04	40.41 ± 0.07	10.46 NS
Creatinine mg/dl	0.835 ± 0.03	0.897 ± 0.03	0.890 ± 0.07	0.116 NS
* (P<0.05), NS: Non-Significant.				

**Table 4**-Distribution of lipid profile according to gender of CVD patients

Parameters	Gender		T-Test
	Male	Female	
Cholesterol (mg/dl)	171.73 ± 6.33	259.56 ± 47.21	48.81 *
Triglyceride (mg/dl)	317.24 ± 13.20	287.44 ± 40.45	64.95 NS
HDL (mg/dl)	35.65 ± 1.12	30.89 ± 2.55	5.90 NS
VLDL (mg/dl)	64.47 ± 2.71	62.00 ± 7.11	13.05 NS
LDL (mg/dl)	93.24 ± 4.96	106.89 ± 13.82	28.81 NS
Urea (mg/dl)	37.09 ± 2.40	29.67 ± 2.92	12.63 NS

**Relationship between lipid profile and PCR results**

The results of the current study revealed that no significant relationship of each HDL, LDL and cholesterol with the existence of *C. pneumoniae* infection in patients ( $P < 0.05$ ), whereas there was positive dramatically increase in triglyceride and VLDL ( $P < 0.05$ ) in positive CVD patients as shown in Table-5.

**Table 5**-Lipid profile in CVD patient according to PCR results

Parameters	PCR results Mean ± SE		T-Test
	Infected with <i>C. pneumoniae</i>	Not infected with <i>C. pneumoniae</i>	
Cholesterol(mg/dl )	189.38 ± 8.28	179.27 ± 13.04	33.81 NS
Triglyceride(mg/dl)	375.34 ± 17.43	276.82 ± 14.65	44.99 *
HDL (mg/dl )	35.69 ± 1.92	34.65 ± 1.21	4.09 NS
VLDL (mg/dl )	76.88 ± 3.46	56.63 ± 2.91	9.04 *
LDL (mg/dl)	97.81 ± 5.71	93.34 ± 6.66	19.96 NS

\* ( $P < 0.05$ ), NS: Non-Significant.

*C. pneumoniae* is involved in respiratory tract infections and to a lower degree in extrapulmonary diseases. Most probable, *C. pneumoniae* is mostly transmitted of human to human by the respiratory tract without need any animal host was detected in 7.7% of patients with CVD in Karbala city\Iraq [12]. Another study done by Abdullah *et al* his results showed that 33.3% of patients were positive for *C. pneumoniae*, while it was negative in all control samples[13]. Davidson *et al* also identified *C. pneumoniae* in the arteries of 37% of all subjects (22/60) [14]. Similarly; from 40 patient and 11 controls, 13 (32.5%) of cases were positive for *16S rRNA* gene of *C. pneumoniae* whereas one of the controls were positive [15]. The present study and other studies suggest the hypothesis that *C. pneumoniae* can be associated with CVD or even consider as a risk factor for atherosclerotic changes. A total number of 90 subjects including of 70 cases with CVD, and 20 healthy control subjects were recruited in this study, the patient ages mean was 54.48 ranged between 30-87, control ages mean was 47.2 ranged between 30-86. The males: females rate was 6:1 (M:F=60:10). The cases were selected from no-hypertension, non-diabetic patient (without any risk factors). According to the results in table (3) Cholesterol showed a significant elevation in less than 50 years group compared with other groups ( $p < 0.05$ ). Also triglyceride was elevated in patients less than 50 years compared with other group, but it was statically not significant. Although there was no significant relationship between HDL VLDL, LDL and age; but on the whole; certain patient with more than 60 years age group have the highest level of HDL VLDL, LDL even urea and creatinine. The level of total cholesterol is excellent predictors of coronary heart disease in these aged lower than 50 years. However, in these ages above 50 years, more precise predictor of coronary heart disease risk were serum lipoprotein measurements, like LDL, VLDL, triglycerides, and HDL and man more effected with CHD than woman with high cholesterol level [16]. Another study recorded, the male was more effected with CHD than female with high cholesterol level [17]. Also a study showed that man more affected than female and plasma levels of cholesterol, LDL and triglycerides were greater in the young paralleled to middle age and elderly patients[18]. Excess intake of saturated fatty acids associated with decreased cereals, fruit and vegetables does not only alter the lipid profile but also increases the risk of coronary disease American Heart Association/American College of Cardiology guidelines recommend adherence to a set of dietary and lifestyle habits including body weight control and physical activity[16]. Low-density lipoprotein cholesterol may be a less important risk factor in

women, perhaps because estrogen protects the arterial wall against LDL deposition [19]. Several studies have shown that high serum concentrations of total and LDL cholesterol and relatively low levels of HDL cholesterol are correlated with development of atherosclerotic lesions and increased cardiovascular risk in men, and thus lowering cholesterol reduces the risk. Low density lipoprotein cholesterol levels rose with age in both men and women [20]. Low-density lipoprotein cholesterol may be a less important risk factor in women, perhaps because estrogen protects the arterial wall against LDL deposition. In the the current study, male appeared to be more affected than females, this finding is in agreement with previous Iraqi studies [21-23], that are in consistence with the former conclusion of Thompson and his colleagues backing the results who stated their female risk of CAD is less than in men because the premenopausal event preserve them against CAD evolution. Sex hormone such as estrogen have capability to assistance lipid metabolism. In addition, women are more likely to have symptoms considered atypical compared with men. There is an urgent need to better understand the presentation of cardiac symptoms in women, in order to facilitate diagnosis and treatment, to initiate aggressive risk factor intervention and to improve the quality of life. [24-26]. Lipid metabolism is coordinated extensively during the host immune response to infection. Lipids act as a part of the host protection, with lipoproteins scavenging for infectious particles such as endotoxin. These events are intermediated by cytokines, such as interleukin-6, TNF- $\alpha$ , interleukin-1, and the interferons. Very-Low-Density Lipoproteins (VLDL) are complexes of lipids and proteins assembled in the liver in response to nutrients and hormones. When VLDL are secreted, they carry almost all of the triglyceride in the blood-stream (they are about 85% triglycerides themselves) [19]. Cytokines can lower lipoprotein lipase action and triglycerides removed and raise VLDL levels [27, 28]. TNF- $\alpha$  prevent the activity of lipoprotein lipase leading to change lipid metabolism, and aggregation of serum triglycerides and a reduction in serum high density lipoprotein cholesterol (HDL-C) [29]. TNF- $\alpha$  raised plasma triglyceride through raise the concentration of free fatty acids, who doing as substrate for triglyceride synthesis, and by decreasing the removing of triglyceride rich lipoproteins (VLDLs) from the circulation [30].

### Conclusion

Approximately one third of CVD patients who enrolled in this study were infected with *C. pneumoniae* whereas no infection recorded within the control group. Also, there was a significant increase of triglyceride and VLDL in patients positive to *C. pneumoniae*; Altogether, the patients with age below 50 years have the highest cholesterol levels, while those with above 60 years have the highest lipoprotein.

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