



The relationship between *Chlamydia pneumoniae* infection and TNF- α in cardiovascular disease patients

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Abstract

Over the last few years the role of microorganisms in the pathogenesis of atherosclerosis has been widely discussed. Advance in basic science have established a fundamental role for inflammation immediating all stages of cardiovascular diseases. *Chlamydia pneumoniae* activates immune cells to produce cytokines such us TNF- α that are important contributor to atherosclerosis. All blood samples were assayed for molecular detection of *Chlamydia pneumoniae* by using conventional polymerase chain reaction (PCR) relying on 16SrRNA and the level of serum TNF- α measured by enzyme linked immunosorbent assay (ELISA). Seventy patients who suffering from CVD (angina, myocardial Infarction and atherosclerosis) aged between 33-86 years have been investigated and compared to twenty of apparently healthy individuals were studies as control group. Twenty six sample (37.14) % detected positive results for *Chlamydia pneumoniae* by PCR techniques in patient group, while all control group were negative, furthermore current study revealed a highly significant elevation ($p < 0.01$) in the mean level of TNF- α in sera of patients with CVD compared to control group. Also there were considerable differences in the level of TNF- α between *Chlamydia pneumoniae* positive and negative within the patient group. The present study concludes there is a significant proportion among patients who infected with *C. Pneumoniae* and these bacteria play an essential role in the pathogenesis of CVD through stimulation of the inflammatory response.

Keywords: *Chlamydia pneumoniae*, cardiovascular disease, TNF- α , PCR.

العلاقة بين العدوى ببكتريا المتدثرات الرئوية وعامل النخر الورمي - ألفا لدى المرضى المصابين بأمراض القلب الوعائية

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الخلاصة

على مدى السنوات القليلة الماضية دور الأحياء المجهرية في التسبب بتصلب الشرايين قد تمت مناقشته على نطاق واسع. وقد بين التقدم في العلوم الأساسية دوراً أساسياً للتهاب الفوري لجميع مراحل أمراض القلب والأوعية الدموية. ببكتريا المتدثرات الرئوية تنشط الخلايا المناعية لإنتاج السيتوكينات مثل عامل النخر الورمي - ألفا التي تساهم بشكل كبير في تصلب الشرايين. تم التحري عن سبعين مريضاً يعانون من أمراض

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القلب الوعائية (الذبحة الصدرية، الجلطة القلبية وتصلب الشرايين) تراوحت اعمارهم بين 33-86 سنة و قورنت النتائج مع عشرون من الاشخاص الاصحاء ظاهريا كمجموعة سيطرة. خضعت جميع نماذج الدم الى التشخيص الجزيئي بهدف التحري عن وجود بكتريا المتدثرات الرئوية باستخدام تقنية تفاعل السلسلة المنكر اعتمادا على وجود الجين 16S rRNA كما تم قياس مستوى عامل النخر الورمي الفا في مصول المرضى والاصحاء باستخدام تقنية الامتزاز المناعي المرتبط بالانظيم. ستة وعشرون عينه دم (37.14%) أظهرت نتيجة ايجابية لبكتريا المتدثرات الرئوية في عينات دم مجموعة المرضى بينما كانت جميع عينات مجموعة السيطرة سالبة لهذا الفحص . فضلا عن ذلك أظهرت نتائج الدراسة الحالية ارتفاعا عالي المعنوية ($p>0.01$) في تركيز عامل النخر الورمي الفا في مصول المرضى بالمقارنة مع مجموعة السيطرة كما لوحظ اختلافا معنويا في مستوى عامل النخر الورمي بين المرضى الذين اظهروا نتيجة ايجابية للبكتريا والمرضى ذوي النتيجة السالبة تستنتج الدراسة الحالية وجود نسبة لا يستهان بها بين المصابين بأمراض القلب الوعائية في المجتمع العراقي مصابين ببكتريا المتدثرات الرئوية وان هذه البكتريا تلعب دورا حيويا في الأمراض من خلال تحفيز الاستجابة الالتهابية.

Introduction

Cardiovascular diseases (CVD) is a type of disorders that involve the blood vessels or heart [1]. It involves coronary artery diseases (CAD) like angina and myocardial infarction (usually famed as a heart attack). Other CVDs involve 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 plausible additional risk factor for atherosclerosis since it is the sole viable pathogen detected in the atherosclerotic plaque [4]. *C. pneumoniae* a gram-negative, intracellular bacterium is characterized from other bacteria by an exceptional life cycle. In its life cycle there are two functionally and morphologically separate cell types: the reproductive reticulate body (RB) and the infectious elementary body (EB) [5]. Chlamydial and human heat shock protein 60 (hsp 60) are stimulate macrophages, endothelial cells and smooth muscle cells, via stimulation of nuclear factor- κ B, prompting appearance of adhesion molecules such as ICAM-1 and VCAM-1 and TNF production [6]. *C. pneumoniae* to play a role in atherosclerotic cardiovascular diseases for its ability to systematically disseminate from the lung through peripheral blood mononuclear cells and to localise in extra pulmonary tissues, such as the vascular wall. Once being inside the vascular tissue, *C. pneumoniae* has been shown to act directly on the cells involved in atherosclerotic process, contributing to endothelial dysfunction, foam cell formation, vascular smooth muscle cell (VSMC) proliferation and migration, and platelet aggregation and induce the elicitation of proinflammatory cytokines like TNF- α , and adhesion molecules, such as ICAM-1 thus contributing to the chronic inflammatory state responsible for the initiation, progression, and destabilisation of atherosclerotic plaque [4,7]. TNF- α is a proinflammatory cytokine with a diversity of biological actions, displayed essential effects on various cell forms that are constituents of atherosclerotic lesions. Each of endothelial cells, macrophages, and smooth muscle cells can produce TNF [8]. High level of TNF- α was linked with the pathogenesis of several situations like myocardial dysfunction and CAD [9]. *C. pneumoniae* stimulate production of cytokines and chemokine's by macrophages which are essential components of atherosclerosis through a Toll-like receptor (TLR) signaling [10]. This work was aimed to evaluate the association between the proinflammatory cytokine TNF- α and *C. Pneumoniae* infections in cardiovascular diseases patients.

Materials and methods

Collection of Blood Sample

Seventy Iraqi patients with CVD (angina, myocardial infraction and atherosclerosis) ranged between 33-86 years were involved in this study. They were confessed to Ibn Al-Bitar specialist center for cardiac surgery in Baghdad between December 2017 and March 2018. They were selected from 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 a permission from requisite ethics committee and informed consents from patients. Patients samples have been

investigated and compared to twenty of apparently healthy volunteers were studied as control group with age ranged between 30-86 years.

Immunological Examination

Tubes containing CAD and control sera were labeled and carried out to measure TNF- α by using ELISA, according to manufacturer's (Shanghai Biological, China for TNF- α) immunological studies achieved in Baghdad Teaching Hospital Laboratories- Ministry of Health- Baghdad.

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

The DNA extraction from blood samples were achieved according to the manual of manufacturer of (Geneaid Company. Korea). A high produce of cleaned DNA be 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 μ l of forward primers, 1.5 μ l of reverse primer, 7 μ l of DNA, 5 μ l of master mix and 10 μ l of nuclease free water, to get a whole volume 25 μ l. In 1.5 % agarose gel, the PCR products were run. Table-1 illustrates The PCR conditions.

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

Gene		Primer sequence	Size of product	PCR condition	Reference
<i>C.pn</i> 16SrRNA	Forward	TGACAACTGTAGAAAT ACAGC	463	95 $^{\circ}$ c 2.5min 1cycle	[11]
	Reverse	ATTTATAGGAGAGAGG CG		94 $^{\circ}$ c 1min 53 $^{\circ}$ c 20sec 30 cycle 72 $^{\circ}$ c 40sec	
				72 $^{\circ}$ C 5min 1 cycle	

Statistical Analysis

The effect of difference factors in study parameters were studied by Statistical Analysis System-SAS 2012 program. For significant comparison between percentage and least significant difference, Chi-square test was used, while LSD or T-Test was used to significant compare between means in this study [12].

Results and Discussion

Molecular detection

DNA bands of *C. pneumoniae* 16SrRNA gene showed that twenty six (37.14%) patients were positive 16SrRNA in their specimens with molecular length 463 base pairs, while all healthy donor ones were negative, and the rate of male was 92.30 % (24/26), while only two of 26 were females 7.69 % as illustrated in Figure-1.

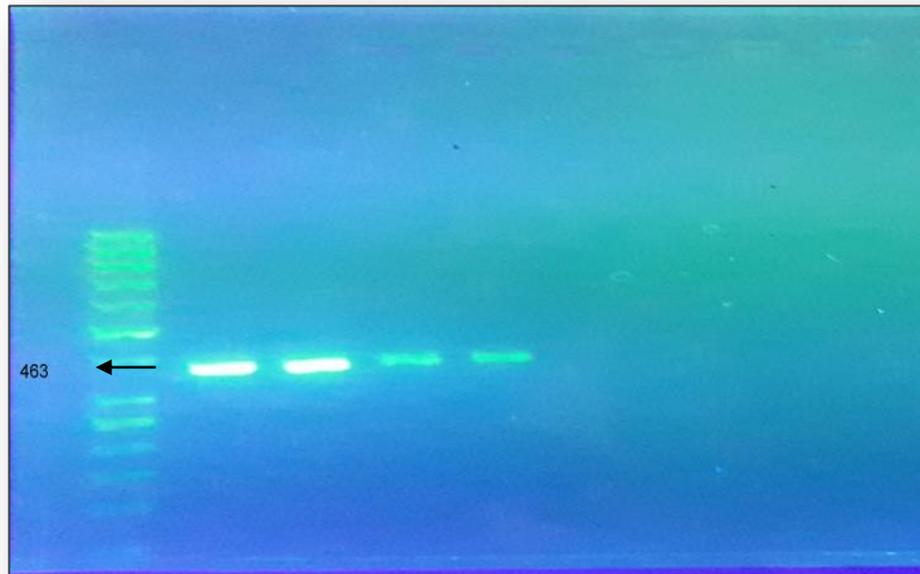


Figure 1-Shows the Gel electrophoresis of amplification PCR product *Chlamydia pneumoniae* specific gene 16SrRNA (1.5% agarose gel) electrophoresed in 75 volt for 2 hrs and photographed under ultraviolet trans illuminator. M: molecular marker (50 bp DNA ladder), stained with red stain bands in the gel

Immunological examination

Serum sample were collected from patients and control were tested for the level of TNF- α by using ELISA. The result showed a highly significant elevation in TNF- α mean concentration of CVD patients compared to healthy donor ones (363.93 ± 23.04), (37.71 ± 1.88) respectively ($p < 0.01$) as shown in Figure-2. Also there were significant differences in the level of TNF- α among CVD patients positive and negative to *C. pneumoniae* (512.96 ± 36.01) and (275.86 ± 20.59) respectively ($p < 0.05$) as illustrated in Figure-3.

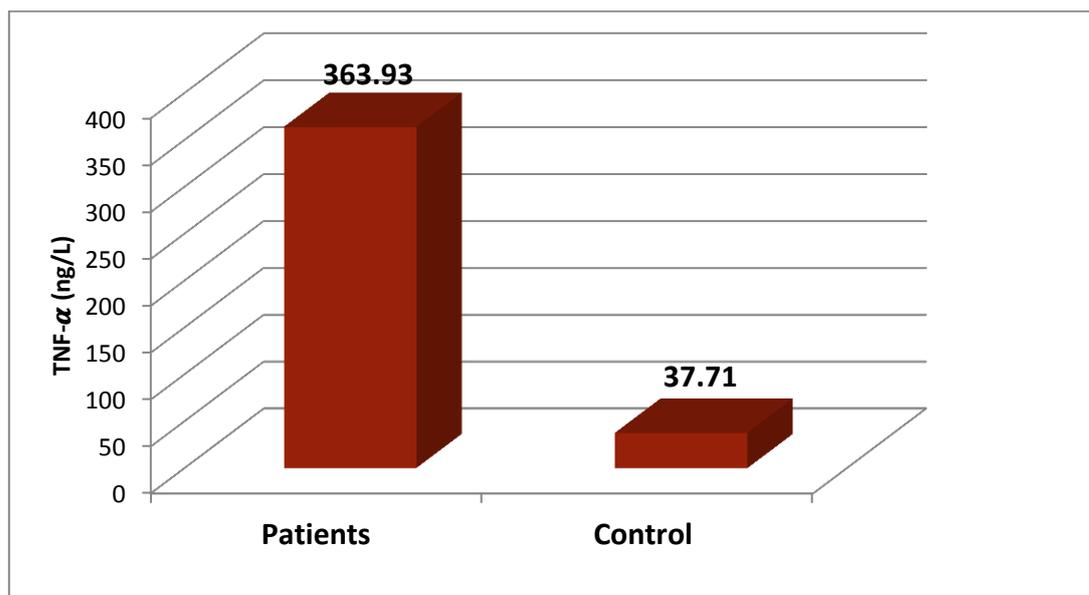


Figure 2-Mean level of TNF- α concentration (ng/L) in sera of CVD patients and control group.

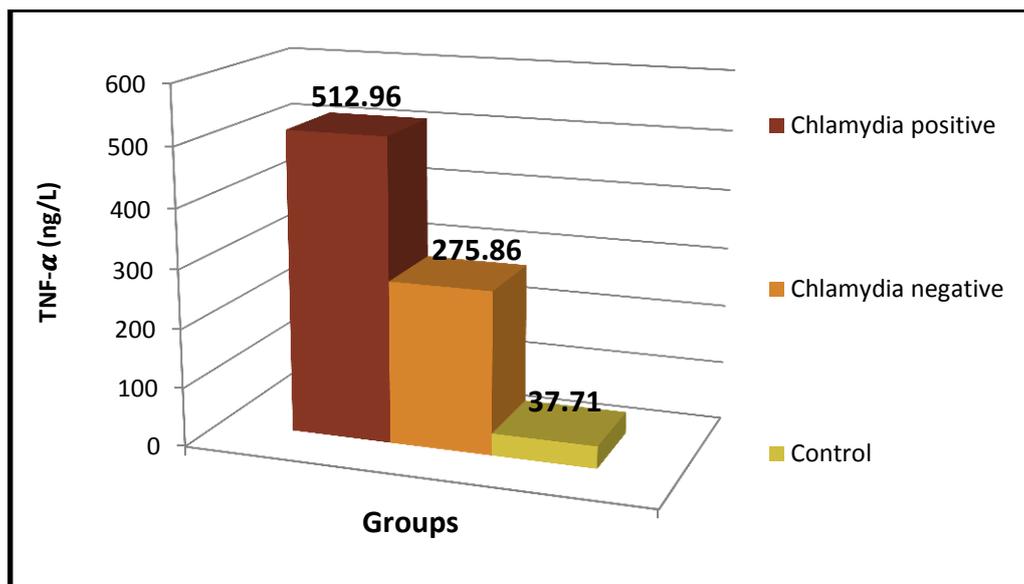


Figure 3- Mean level of TNF- α concentration (ng/L) in sera of CVD patient positive and negative to *C. pneumoniae* and control group.

These results were corresponded with local study, in which DNA of *C. pneumoniae* was detected in 7.7% of patients with CVD in Karbala city\Iraq[13]. Another study done by Abdullah *et al* his results showed that (33.3%) of patient were positive for *C. pneumoniae*, while it was negative in all control samples[14]. Davidson *et al* also identified *C. pneumoniae* in the arteries of 37% of all subjects (22/60)[15]. Similarly; from 40 patient and 11 controls, 13 (32.5%) of cases were positive for 16SrRNA gene of *C. pneumoniae* whereas one of the controls were positive[16]. This study and other studies suggest the hypothesis that *C. pneumoniae* can be associated with CVD or even consider it as a risk factor for atherosclerotic changes[17]. Infection with *C. Pneumoniae* plays an essential role in atherosclerotic progression in coronary arteries reflecting a deleterious effect of this bacterium or its consequences like endotoxins, cytokines, etc. on the coronary endothelium[18]. TNF- α is a proinflammatory cytokine that impact on endothelial dysfunction, contributes to plaque rupture and adhesion molecule expression blood coagulation [8]. TNF α is a powerful inducer of local inflammation. It increases permeability of the endothelial cell barrier, promotes the expression of leukocyte adhesion molecules via nuclear factor-kappa B and increases the uptake of macrophages in atherosclerotic lesions .so it is directly promoting in atherosclerosis. [19]Increased levels of TNF- α were recorded in CAD and atherosclerotic patients [7, 20]. It could be used as predictor for CAD in Koreans[21]. Elevated levels of TNF- α were recorded in CVD patients who Chlamydia lipopolysaccharide IgA test are positive [22]. In spite of acting polymorphisms of TNF- α as genetic risk factors in developing CAD [23], some of reports were unable to find a considerable relationship binding CVD with TNF- α gene polymorphisms [24], as in between TNF- α polymorphism and MI/CVD in Caucasian and Asian populations[25]. Interestingly, *Chlamydia* has influence on endothelial cells and vascular smooth muscle cells, mediated through cytokines like TNF- α resulting in vascular smooth muscle proliferation ,up regulation of inflammation ,endothelial apoptosis[16]. Moreover, Chlamydial hsp60 centralize in human atheroma and responsible of macrophage and TNF regulation as well as matrix metalloproteinase (MMPs) expression [26]. Localization of hsp60 and *Chlamydia* hsp60 in macrophages was recognized. Both hsp forms stimulate proinflammatory cytokines like TNF- α and metalloproteinase[27]. TNF- α affects hypertriglyceridemia and lipid metabolism by lessening lipoprotein lipase action in adipose tissue. It related with elevation of triglycerides and enhance liver fatty acid formation[28].

Conclusion

About one third of Iraqi CVD patients in this study were infected with *C. pneumoniae* with significant elevated levels in serum TNF- α , this may reflect a possible role of the bacterium in the pathogenesis of CVD through stimulation of the inflammatory response.

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