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Original article

Chlamydia pneumoniae heat shock protein 60 is associated with apoptotic signaling pathway in human atheromatous plaques of coronary artery disease patients

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KEYWORDS
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Human atherosclerosis

Summary
Background: Chlamydia pneumoniae heat shock protein (HSP) 60 is known to contribute to the activation of inflammation. In addition, there are contradictory reports on *C. pneumoniae* and their role in activation of pathways (apoptotic/antiapoptotic/necrosis) in coronary artery disease (CAD). Hence, more studies are required to know the actual role of *C. pneumoniae* in activation of apoptotic/antiapoptotic/necrosis pathways.
Methods and results: In this study, two sets of patient groups (cHSP60 positive and cHSP60 negative) were included and gene expression was studied by cDNA micro array and real time polymerase chain reaction arrays. Expression of *Caspase*-3, 8, 9, *c-FLIP*, *PPAR-*γ, *PGC*-1α, and *Gsk*-3b were also evaluated at protein level by immunoblotting. In cHSP60 positive CAD patients significantly higher ($p < 0.001$) mRNA expression was found for *CCL4*, *CXCL4*, *CXCL9*, *IL-8*, *CD40LG*, *CD8*, *TGF*β*1*, *TGF*β*2*, *APOE*, *EGR1*, *CTGF*, *APOB*, *LDLR*, *LPA*, and *LPL*, whereas significantly lower ($p < 0.001$) mRNA expression was detected for *CD4*, *IL1F10*, *IFNA2*, and *IL-10* as compared to cHSP60 negative CAD patients. Additionally, at protein level expression of *Caspase*-3 ($p = 0.027$), 8 ($p = 0.028$), and 9 ($p = 0.037$) were higher and *c-FLIP* ($p = 0.028$) and *PPAR-*γ ($p = 0.95$) expression were comparable in cHSP60 positive CAD patients compared to cHSP60 negative CAD patients.

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Conclusion: Genes/proteins of pre-apoptotic caspase dependent/independent pathways, chemokines, and inflammatory cytokines receptors were significantly up-regulated in human atheromatous plaques of cHSP60 positive CAD patients suggesting an association of cHSP60 with CAD.
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Introduction

The high diversity of bacterial DNA in coronary lesions supports the infection hypothesis in the pathophysiology of coronary atherosclerosis. The prevalence of *Chlamydia pneumoniae* has been reported to be between 0% and 100% in human plaques by using various detection methods [1—5]. *C. pneumoniae* has been detected frequently in lesions of the aorta, iliac, carotid, and coronary arteries, whereas it has been rarely found in normal arterial tissue [5,6]. Further, clinical presentation, risk factors, and inflammatory status, but not age, are important factors for plaque components [7]. Viable *C. pneumoniae* has been cultured from a small proportion of atherosclerotic plaques [8]. Further, in vitro study suggests that *C. pneumoniae* is capable of infecting three cellular components of the human vascular wall — namely endothelial cells, smooth muscle cells (SMCs), and macrophages and can proliferate in these components [9].

A heat-stable component of *C. pneumoniae* induces macrophage foam cell formation and also stimulates oxidation of low-density lipoprotein (LDL) [10,11]. Moreover, molecular mimicry between *C. pneumoniae* heat shock protein (HSP) 60 antigens and human HSP60 has been reported and also suggests a role in the activation of inflammation [12]. Streblow et al. reported that accumulation of lipids is thought to be both an initiator and accelerator for atherosclerosis plaque formation [13,14]. Additionally, *C. pneumoniae* has been reported to alter the lipid metabolism of infected SMCs and macrophages [13]. Also, *C. pneumoniae* infection increases endothelial expression of adhesion molecules, which significantly enhances trans endothelial migration of inflammatory cells [15,16]. These inflammatory processes are orchestrated in part by chemokines, which participate in the inflammatory process by mediating monocyte recruitment to the sites of injury, vascular SMC proliferation, neovascularization, and platelet activation [17]. Peroxisome proliferator-activated receptor-γ (PPARγ) regulates lipid, lipoprotein metabolism, and glucose homeostasis and is up regulated after *C. pneumoniae* infection [18,19]. Moreover, caspases are present in nucleated cells as inactive zymogens (pro-caspases) and get activated in apoptotic pathways [20—22]. Two tiers of different caspases are consecutively activated, such as signal, initiator caspases (mainly caspase-8 and 9) and effector caspases (caspase-3) [20]. Cytotoxicity associated with chlamydial infection is well recognized and has been linked to induction of apoptosis [21]. However, controversy exists as to the nature of the apoptotic mechanisms, and more than one route for cell death may be utilized depending on the host cell type involved, epithelial cell versus macrophage [21].

There are contradictory reports on *C. pneumonia*-induced activation of apoptotic/antiapoptotic pathways; even some researchers hypothesized that *C. pneumoniae* may induce necrosis in host cells. The reason for these controversial results may be because these studies have been conducted on different cell lines and their modes of activation could therefore vary [20,23,24]. Further, in order to get a real picture, human studies are required to know the role of *C. pneumoniae* on activation of signaling pathways in CAD. Hence, for the first time we have performed this study on human atherosclerotic plaques and evaluated signaling pathways including atherosclerosis related genes, cytokine and chemokine receptor genes, apoptosis and antiapoptotis-related genes at RNA level using micro array and polymerase chain reaction (PCR) arrays and protein levels by immunoblotting in cHSP60 positive and negative CAD patients.

Materials and methods

Unless otherwise stated, all the reagents were purchased from Sigma Aldrich (Saint Louis, MO, USA) and antibodies from Cell Signaling (Cell Signaling Technology, Beverly, MA, USA). Plastic wares and glass wares for all tissue-related works were obtained from Greiner Bio-one, Frickenhausen, Germany.

Patient enrollment, atheroma collection and handling

Forty patients (28 men, 12 women) mean age 51 ± 13 years attending the Department Of Cardiothoracic & Vascular Surgery, Safdarjung Hospital, New Delhi from September 2007 to April 2008 were enrolled in the study. Prior informed written consent was obtained from each patient. The study received clearance from the ethical committee, Safdarjung Hospital. Atheromatous tissues (coronary artery) were collected in aseptic conditions and placed in 30 ml of transport medium immediately upon resection. The detailed procedure of atheroma collection and handling are as described earlier [25].

Atheroma histology, DNA and RNA isolation and testing

Histological examination of atheromatous tissues by hematoxylin and eosin staining revealed lipid core, a lesion with fibrosis, large areas of calcification with infiltration of SMCs, endothelial cells, macrophages, and lymphocytes. Total DNA was isolated form atheromatous plaques and was checked for the positivity for *C. pneumoniae*, *Helicobacter pylori*, cytomegalovirus (CMV), and herpes simplex virus-1 (HSV-1) using multiplex real time PCR as described earlier [26]. Total RNA from the coronary artery atheroma samples was

Table 1 Baseline clinical characteristics of cHSP60 positive and cHSP60 negative coronary artery disease patients.

Baseline characteristics	cHSP60 positive ($n = 18$)	cHSP60 negative ($n = 22$)	p-value
Age (years)	53 ± 11	50 ± 12	NS
Male	12 (66.6%)	14 (63.6%)	NS
Female	05 (27.7%)	07 (31.8%)	NS
HT	13 (72.2%)	12 (54.5%)	NS
DM	07 (38.8%)	04 (18.2%)	NS
Smoking	13 (72.2%)	12 (54.5%)	NS
Non-alcoholic	16 (88.8%)	16 (72.7%)	NS
SLS	12 (66.6%)	11 (50%)	NS

cHSP, *Clamydia pneumoniae* heat shock protein; HT, hypertension; DM, diabetes mellitus; SLS, sedentary lifestyle; NS, non significant.

isolated using RNeasy fibrous tissue mini kit (Qiagen Sciences, Germantown, MD, USA) and the concentration was determined by a RNA dye-binding assay (Pico-Green, Molecular Probes, Eugene, OR, USA). For cDNA synthesis, RETRO script (Ambion Inc., Austin, TX, USA) was used as per manufacturer's instructions.

Classification of cHSP60 positive and negative CAD patients

The clinical characteristics of cHSP60 positive and cHSP60 negative CAD patients are presented in Table 1. Monoplex reverse transcriptase (RT) PCR was performed for detecting positivity for *C. pneumoniae* using *C. pneumoniae* HSP60 gene in CAD patients who were earlier detected positive for *C. pneumoniae* specific 16S rRNA. Primers and probes used for amplification and checking specificity were custom designed (Applied Biosystems, Foster City, CA, USA). Further, study was performed only on cHSP60 positive ($n = 5$) (negative for *H. pylori*, CMV, and HSV-1) and cHSP60 negative ($n = 4$) (also negative for *H. pylori*, CMV, and HSV-1) CAD patients.

Microarray experiment

Customized services for microarray were performed at Ocimum Biosolution (Hyderabad, India). There were 40 K human genes spotted on slides and hybridized with Cy3 and Cy5 dye in single color. Atheromatous plaques from coronary artery obtained from CAD patients who were positive for *C. pneumoniae* only (*H. pylori* and CMV negative) were treated as test samples and those obtained from CAD patients negative for *C. pneumoniae* (*H. pylori* and CMV negative) were treated as control samples. All experimental procedures (including hybridization, scanning) were performed at Ocimum laboratory followed by data analysis through bioinformatics approach. Detailed protocol is available on web portal, GEO accession number: GSE19590. Further, we validated these data by real time RT-PCR using PCR arrays at RNA level and using immunoblotting at protein levels.

Quantitative real time RT-PCR arrays

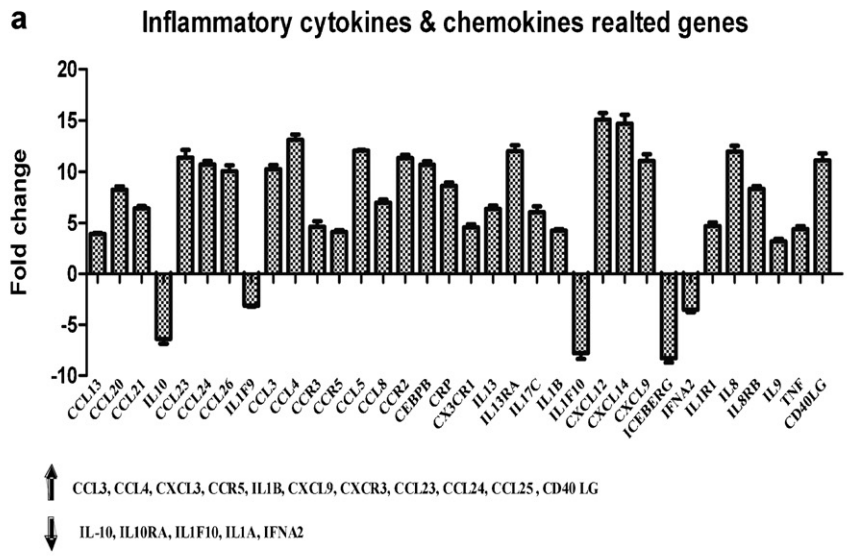
Gene expression was studied using inflammatory cytokine and receptor PCR array and atherosclerosis PCR array — 'focused gene expression profiling PCR array' for human as per manufacturer's instructions (SABiosciences Corporation, Frederick, MD, USA). All information about quantitative real time RT PCR arrays was previously reported [25].

Immunoblotting

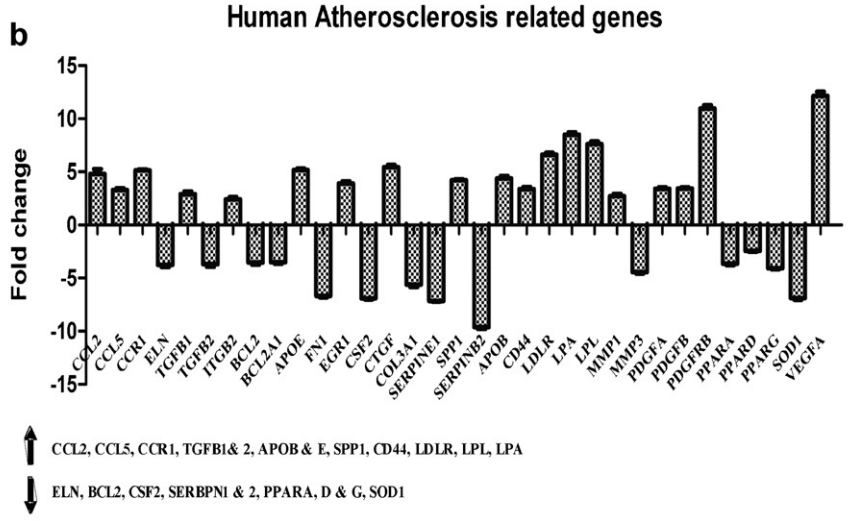
One piece of atheromatous plaque (coronary artery) was homogenized through mortar-pestle with the help of liquid nitrogen and subsequently treated with lysis buffer [0.5% Nonidet P-40, 150 mM NaCl, 0.1% sodium dodecyl sulfate (SDS), 50 mM NaF, 1 mM Na_3VO_4, and 1 mM phenyl methyl sulfonyl fluoride] containing the complete protease-inhibitor cocktail (Roche Diagnostics, Mannheim, Germany). Protein concentration was determined by the Bradford protein assay (BioRad Laboratories, Hercules, CA, USA) with BSA as standard. Extracted proteins (60 μg) were electrophoresed on 8—12% SDS polyacrylamide gels and transferred to polyvinyl difluoride membranes (BioRad Laboratories). The membranes were reversibly stained with ponceau S (Sigma Aldrich) to confirm complete transfer. Membranes were blocked with 5% nonfat dry milk in PBS-Tween-20 and incubated with rabbit polyclonal anti-IgGs against *Caspase-3*, *8*, *9*, *c-FLIP*, *PPAR-*γ, *PGC-1*α, *Gsk3b*, and *beta actin* and further incubated with the monoclonal goat anti-rabbit IgG conjugated with horseradish peroxidase. Subsequently, they were developed using diaminobenzamide as the detection agent and analyzed using the Image J software (NIH, Bethesda, MD, USA) (http://rsbweb.nih.gov/ij/index.html). Representative blots are shown in the results section.

Statistical analysis

SPSS version 12.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical testing. Continuous variables were analyzed with the two-sample t test (in the case of normal distribution) or the Mann—Whitney U rank-sum test. Simultaneously, an alpha level of 0.05 was set as the level of significance. Image J software was used for the calculation of immunoblot band area in all groups. Analysis of microarray data was performed at Ocimum Biosolution.



CCL3=Chemokine (C-C) motif ligand 3, CXCR3= Chemokine (C-X3-C motif) receptor 3, CCR5=Chemokine (C-C motif) receptor 5, CXCL9= Chemokine (C-X-C motif) ligand 9, CD40LG= CD40 ligand (TNF superfamily, member 5, hyper-IgM syndrome), IL10RA= Interleukin 10 receptor-alpha, IL1A= Interleukin 1, alpha, IFNA2= Interferon, alpha 2



APOB=Apolipoprotein B, BCL2=B-Cell CLL/lymphoma 2, CSF2= Colony stimulating factor 1 (macrophage), ELN=Elastin, LDLR= Low density lipoprotein receptor, LPA= Lipoprotein, LPL= Lipoprotein lipase, PPARA= Peroxisome proliferative activated receptor-alpha, SERPINE1= Serine peptidase inhibitor, clade B-member 2, SOD1= Superoxide dismutase 1-soluble, TGFB1= Transforming growth factor, beta 1

Figure 1 (a) Human inflammatory cytokine and chemokine receptor genes in cHSP60 positive and negative coronary artery disease patients. (b) Human atherosclerosis signaling genes in cHSP60 positive and negative coronary artery disease patients. cHSP, *Clamydia pneumoniae* heat shock protein.

Results

Microarray experiment on human 40K genes in cHSP60 positive and negative CAD patients

There were 437 genes which were up regulated and 330 genes were down regulated in cHSP60 positive patients compared to cHSP60 negative CAD patients. *GMCSF*, *LPL*, *TGF-β receptor*, *ICAM1 receptor*, *IL-8*, *NF-kB*, *APOA1* and *VEGF* were significantly ($p < 0.001$) up regulated in cHSP60 positive CAD patients compared to cHSP60 negative CAD patients (Table 2).

Real time RT-PCR arrays

Two constitutive genes (B2-microtubulin and beta actin) were used in all experiments as uniform expression pattern was observed for both the genes (out of 5

Table 2 Comparison of *Clamydia pneumoniae* heat shock protein 60 positive and negative in micro array and polymerase chain reaction array (PCR) experiments.

S.No.	Genes	Micro array		PCR array	
		Up-regulated	Down-regulated	Up-regulated	Down-regulated
1	GMCSF	6.6		2.9	
2	LPL	6.6		6.6	
3	SMAD	5.3			9
4	TGF-β receptor	4.5		5.2	
5	TNFAIP3	3.64		4.2	
6	ICAM-1 receptor	9.5		5.2	
7	IL-8	9.4		11.8	
8	TNF-α	9.4		4.3	
9	IL-1	6.6		4.3	
10	NF-kB	5.2		5.3	
11	PRDX1	3.6		12.2	
12	GABA	3.2		11	
13	VEGF	9.2			3.6
14	APOA	6.4		4.2	
15	COL9A1	7.8		4.8	
16	BRCA1		5.4		8.1
17	TP72		2.8		3.2

GMCSF, granulocyte-macrophage colony-stimulating factor; LPL, lipoprotein lipase; SMAD, *Caenorhabditis elegans* mothers against decapentaplegic; TGF-β, transforming growth factor beta; TNFAIP3, tumor necrosis factor, alpha-induced protein 3; ICAM-1, intercellular adhesion molecule 1; IL-8, interleukin-8; NF-Kb, nuclear factor kappa-light-chain; PRDX1, peroxiredoxin-1; GABA, γ-aminobutyric acid; VEGF, vascular endothelial growth factor; APOA, apolipoprotein A-I; COL9A, vascular endothelial growth factor; BRCA, collagen alpha-1(IX) chain.

constitutive genes) in cHSP60 positive and negative CAD patients.

a) Human inflammatory cytokines and receptors PCR array genes in cHSP60 positive and negative CAD patients

In our study *CCL2*, *CCL3*, *CCL4*, *CCL5*, *CCL20*, *CCL21*, *CCL23*, *CCL24*, *CEBPB*, *CRP*, *IL-13RA*, *CXCL12*, *CXCL1*, *CXCL9*, *IL-8*, *CD40LG* were significantly ($p < 0.001$) up-regulated while *IL1F10* and *IFNA2* were significantly ($p < 0.001$) down regulated in cHSP60 positive CAD patients compared to cHSP60 negative CAD patients (Fig. 1a).

b) Human atherosclerosis PCR array genes in cHSP60 positive and negative CAD patients

CCL2, *CCL5*, *TGFB1*, *TGFB2*, *APOE*, *EGR1*, *CTGF*, *APOB*, *LDLR*, *LPA*, *LPL*, *PDGFRB*, and *VEGFA* were significantly ($p < 0.001$) up regulated while *ELN*, *CSF2*, *SERP1NB2*, and *SOD1* were significantly down regulated ($p < 0.001$) in cHSP60 positive CAD patients compared to cHSP60 negative CAD patients (Fig. 1b).

Protein expression profiles in cHSP60 positive and negative CAD patients

Beta actin was used for normalization of protein profile expression in all experiments. Expression of *Caspase-3* ($p = 0.027$), *8* ($p = 0.028$), and *9* ($p = 0.037$) were higher while expression of *c-FLIP* ($p = 0.028$) was lower and expression of *PPAR* ($p = 0.95$) was comparable in cHSP60 positive CAD patients compared to cHSP60 negative CAD patients. Expression of *PGC-1α* ($p = 0.026$), and *Gsk3b* ($p = 0.024$) was also higher in cHSP60 positive CAD patients compared to cHSP60 negative CAD patients (Figs. 2—5).

Discussion

C. pneumoniae infection causes increased LDL uptake in macrophages, with a subsequent increase in foam cell formation [26]. The ability of pathogens to increase lipid accumulation in macrophages and SMCs is an important step in accelerating atherosclerosis [13,27]. *C. pneumoniae* is able to disseminate via the circulation throughout the body within monocytes and through this way it can enter atherosclerotic lesions [26]. Earlier we reported higher antibody levels of *C. pneumoniae* IgA in the sera of CAD patients [28], and also detected higher positivity for *C. pneumoniae* in comparison to other pathogens in atheromatous plaques of CAD patients using real time PCR [25]. Infection of monocytes with *C. pneumoniae* increases adherence of infected monocytes to endothelial cells [29] and promotes LDL oxidation [30], resulting in accelerated uptake of cholesterol by macrophages and subsequent foam cell formation [26]. Increased LDL oxidation was also observed upon infection of endothelial cells with *C. pneumoniae* [26]. In this study we have shown that CAD patients positive for cHSP60 have higher levels of lipid transporter (*APO-A*, *B*, and *E*) and lipid signaling molecules (*LDLR*, *LPL*, *LPA*) compared to cHSP60 negative CAD patients. Microarray and PCR array results support the enhancement of VEGFA and other lipid transporters as well as lipid signaling genes in cHSP60 positive CAD patients. Monocyte recruitment into

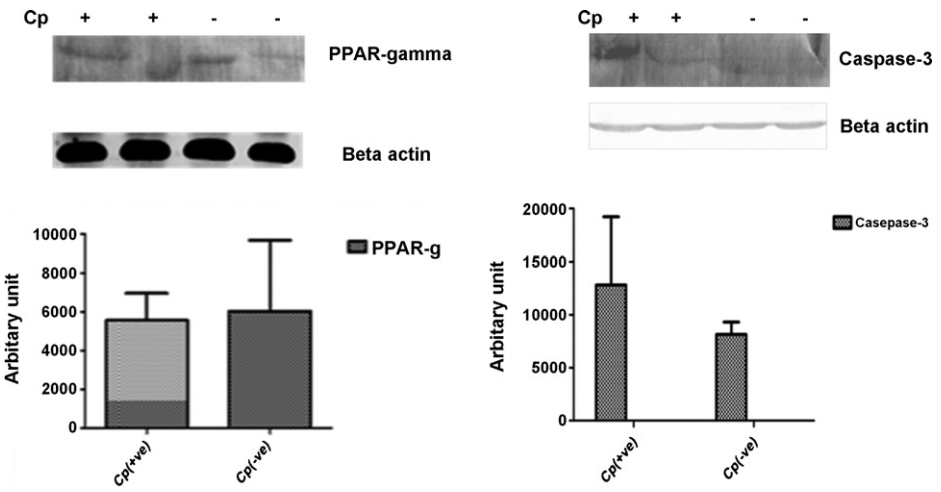


Figure 2 Immunoblotting of PPAR-gamma and caspase-3 in cHSP60 positive and negative CAD patients. PPAR, peroxisome proliferator-activated receptor; cHSP, *Clamydia pneumoniae* heat shock protein; Cp, *Clamydia pneumonia*.

the arterial wall in response to injury is a multistep process that involves reversible adhesion of monocytes to the endothelium, activation of monocytes, firm adherence, and, finally, migration to the sub endothelial space through endothelial cell junctions [31]. A higher level of *VEGFA* might be associated with these multistep processes [32]. Therefore, whether LDL-loaded macrophages with *C. pneumoniae* infection have any effect on cell death and, if so, whether this death occurs by apoptotic/necrotic, caspase-dependent/independent pathways were investigated in our study. Apoptosis is a highly regulated cellular process that consists of diverse upstream private pathways for transducing extracellular death signals into intracellular events and a common downstream effector pathway for amplification of caspases [33]. In human atherosclerotic lesions, enhanced expression of cHSP60 has been detected [34]. Despite the inhibitory effect that *Chlamydia* infection has on apoptosis induced by various agents, some findings suggest a putative role for caspase-dependent apoptosis in spreading infection [35]. On the other hand, it has been reported that chlamydiae are capable of inducing cell death via caspase-independent pathways [36]. In our study, levels of *caspase 3*, *8*, and *9* were higher whereas levels of *c-FLIP* and *PPAR-*α and *-*γ were lower in cHSP60 positive CAD patients. Earlier Dean et al. and Fischer et al. had reported that *C. pneumoniae* infection down regulates pro-apoptotic cytoplasmic proteins such as *caspase-3* and *cytochrome c* [23,37]. Moreover, it has been found that the regulatory *caspase-8* is directly activated by death receptors, whereas *caspase-9* activation follows mitochondrial stress [38]. Both pathways merge by activating executioner *caspase-3* [39]. It has been suggested that different proapoptotic and anti-apoptotic proteins participate in the regulation of apoptosis [40].

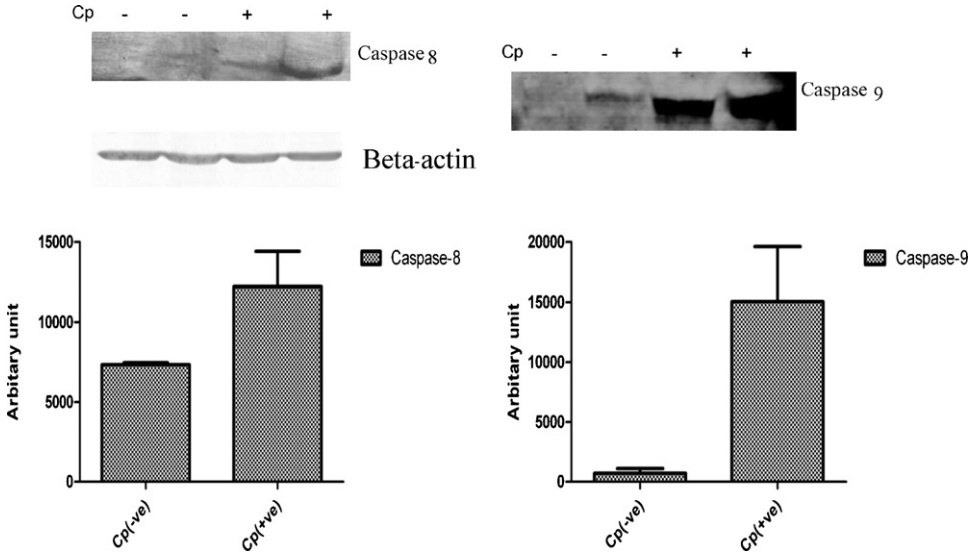


Figure 3 Immunoblotting of caspase-8 and caspase-9 in cHSP60 positive and negative coronary artery disease patients. cHSP, *Clamydia pneumoniae* heat shock protein; Cp, *Clamydia pneumonia*.

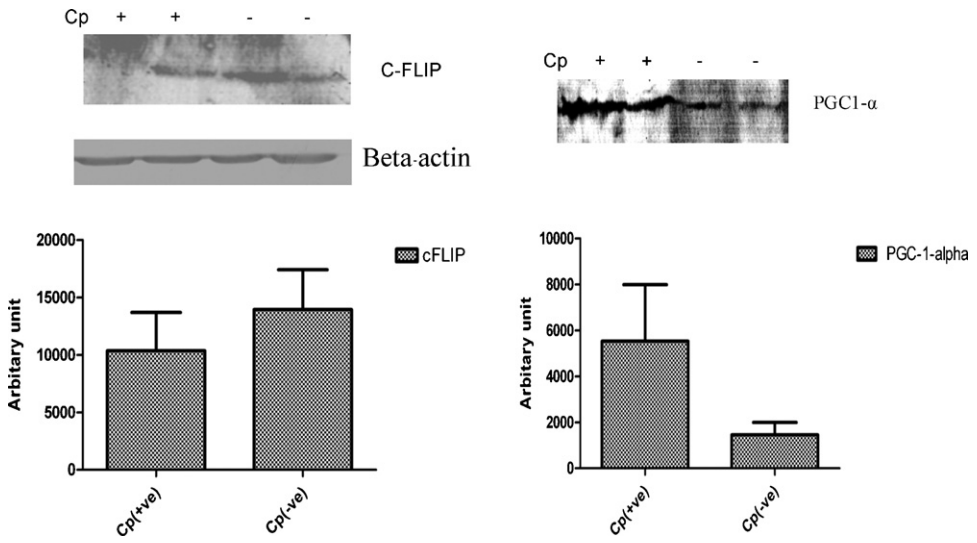


Figure 4 Immunoblotting of c-FLIP and PGC1-α in cHSP60 positive and negative coronary artery disease patients. cHSP, *Clamydia pneumoniae* heat shock protein; Cp, *Clamydia pneumonia*.

Hence, this study reveals that cHSP60 positive CAD patients show higher expression of pre apoptotic rather than necrotic-related genes or proteins. Further, expression of genes or proteins related to both caspase dependent and independent pathways were higher in cHSP60 positive CAD patients compared to cHSP60 negative CAD patients. In contrast to this, Hauer et al. reported that both live and inactivated forms of *C. pneumoniae* induce a necrotic form of cell death which augments the apoptotic cell death induced by the accumulation of oxidized LDL by macrophages [26]. Also Dean et al. hypothesized that *C. pneumoniae* infection activates antiapoptotic proteins [37]. Additionally, Fischer et al. suggested that *Chlamydia* can both induce and inhibit apoptosis [23]. It is easily conceivable that chlamydial infection can result in apoptosis in one constellation of cell type and bacterial strain but not in other combinations [20]. Further, it has been reported that *Chlamydia* infection of an organ can lead to a local immune response followed by systemic activation of auto reactive T and B lymphocytes [41]. T cells, macrophages, and mast cells infiltrate the lesion and are particularly abundant in the shoulder region where the atheroma grows [42]. In our study, expression of CD8 was higher while expression of CD4 was lower in cHSP60 positive CAD patients. Moreover, CD8+ T cells restricted by major histocompatability complex class I antigens are also present in atherosclerotic lesions [43]. Halme et al. suggested that *C. pneumoniae*-induced T-cell activation seemed to be linked with CD8 cells during the active stage of infection [44]. Also, Loomis et al. reported that CD8+ T cells play a critical role in protection against most intracellular pathogens, including *Chlamydia* [45].

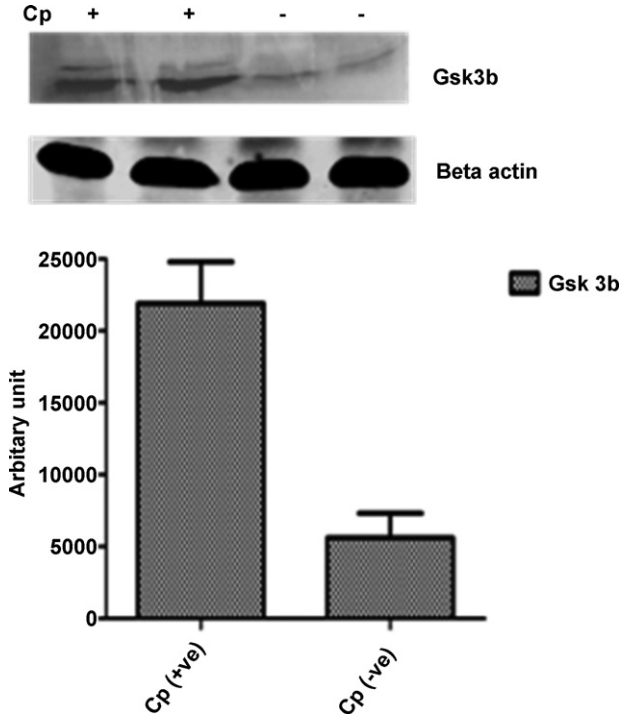


Figure 5 Immunoblotting of Gsk3b in cHSP60 positive and negative coronary artery disease patients. cHSP, *Clamydia pneumoniae* heat shock protein; Cp, *Clamydia pneumonia*.

T-Bet has been identified as a Th1 cell-specific factor that induces the production of *IFN-γ* by developing Th2 cells [46]. GATA3 is a zinc-finger transcription factor and is crucial for inducing key attributes of Th2 cells including transcriptional competence of the Th2 cytokine cluster, which includes the genes encoding *IL-13*, *IL-4*, and *IL-5* [47]. Again in this study we found that CAD patients with atheromatous plaques support the Th2-mediated response as expression of *GATA3* is higher and T-Bet is lower in cHSP60 positive CAD patients. Earlier in our study, a similar pattern was found in serum of CAD patients [48]. Pro-inflammatory molecules are actively involved in the activation and migration of leukocytes to sites of vascular injury and inflammation [49]. Our study also demonstrates that cHSP60 positive CAD patients have higher expression of cytokine and chemokine receptors. Expression of IL-8, TGF-β receptor, ICAM1 receptor, *TNF-α*, *IL-1*, *NF-kB*, *CCL3*, *CCL4*, *CXCL3*, *CXCL4*, *CCR5*, *IL1B*, *CXCL9*, *CXCR3*,

CCL23, *CCL24*, and *CCL25* were higher whereas expression of *IL-10*, *IL-10RA*, *IL1F10*, *IL1A*, and *IFNA2* were lower in cHSP60 positive CAD patients. Although C. pneumoniae is known to preferentially infect the epithelial tissue of the respiratory tract, this bacterium can also multiply in vitro in monocytes/macrophages, T lymphocytes, endothelial cells, and aortic SMCs [50]. Earlier Mueller et al. reported that *CXCL4* can induce signaling in activated T lymphocytes, which results in their chemotactic migration and also suggested a role of *CXCL4* in T cell-mediated immunoregulation [51]. It has also been reported that *CXCL4* can induce differential regulation of the transcription factors like *T-bet* and *GATA-3*, suggesting an ability to modulate Th1/Th2 polarization [52]. *CXCR7* is a highly conserved chemokine receptor that binds with high affinity to the chemokine *CXCL12* [53]. Similar to the other *CXCL12 receptor CXCR4*, *CXCR7* are widely expressed and play a role in fetal development [54].

During the course of a bacterial infection, the bacterial DNA acts as a potent adjuvant facilitating the activation of auto aggressive T cells [55]. The macrophages may adhere to coronary vessels, for example, where they can cause chronic cytokine-mediated inflammatory reactions inflicting direct endothelial damage [56]. There is evidence of molecular mimicry between bacterial antigens and heart specific proteins indicating that bacterial peptides can trigger tissue-specific inflammation of the heart [57]. Lipid-loaded macrophages (foam cells) are a major cellular component of atherosclerotic lesions and chronic infection of foam cells with *C. pneumoniae* could exacerbate the inflammatory response which is associated with the initiation and progression of atherosclerotic lesions [58]. Earlier correlative studies have supported a possible link between atherosclerosis and chronic or persistent infection of *C. pneumoniae* [59].

Earlier records and literature [60] point towards an inflammatory basis and potential etiological role for various infective agents, specifically *C. pneumoniae*, in the pathogenesis of atherosclerosis. From animal and human pathological specimen examinations, micro-organisms are found to exist preferentially in atheromatous tissues. *C. pneumoniae* has been shown to promote the process of atherosclerosis through a variety of immunological mechanisms. Another possible mechanism of damage is that *C. pneumoniae* infection may stimulate an increase in tissue factor activity and platelet adhesion, and thus promote thrombogenicity. Infection in monocytes or macrophages, endothelial cells and vascular SMCs have been shown to induce pro-inflammatory and pro-coagulant protein production (tissue factor, plasminogen activator inhibitor-1, MCP-1), through the activation of nuclear trancription factors such as NF-kB in these cells. Further, Chlamydial hsp 60 could also activate macrophage TNF-α and matrix metalloproteinases, which are enzymes that can cause connective tissue degradation and atherosclerotic plaque rupture. Viable *C. pneumoniae* have also been cultured from atheromatous plaques, suggesting a more causal relationship. In contrast, *C. pneumoniae* antigens were not detectable in normal arterial walls or non-atherosclerotic arterial segments in people known to have atherosclerosis. Hence, benefits of the secondary prevention of atherosclerosis have been demonstrated in some antibiotic intervention studies for *C. pneumoniae*.

In conclusion, in this study using human atheromatous plaque at RNA and protein levels, it is demonstrated that genes/proteins of pre apoptotic caspase-dependent/independent pathways, chemokine, and inflammatory cytokine receptors were upregulated in cHSP60 positive CAD patients showing cHSP60 association with CAD and suggests its role in progression of CAD. The limitation of the present study is that using homogenized tissue samples only the gene and protein expressions of various molecules related to cell death signaling and inflammation were evaluated. Therefore, it is uncertain in which cell types the death signaling is activated. In addition, protein expressions of certain enzymes such as caspases do not always represent the enzyme activities.

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