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
Caspase; Pre-apoptotic; Necrosis; Lipid; Inflammatory cytokines; 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 (chHSP60 positive and chHSP60 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-\(\gamma\), PGC-1\(\alpha\), and Gsk-3b were also evaluated at protein level by immunoblotting. In chHSP60 positive CAD patients significantly higher (\(p<0.001\)) mRNA expression was found for CCL4, CXCL4, CXCL9, IL-8, CD40LG, CD8, TGF\(\beta\)1, TGF\(\beta\)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 chHSP60 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-\(\gamma\) (\(p=0.95\)) expression were comparable in chHSP60 positive CAD patients compared to chHSP60 negative CAD patients.

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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 antiapoptosis-related genes at RNA level using micro array and polymerase chain reaction (PCR) arrays and protein levels by immunoblotting in chSHP60 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, Frickhausen, 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
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, RETROscript (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.

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>cHSP60 positive (n=18)</th>
<th>cHSP60 negative (n=22)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>53 ± 11</td>
<td>50 ± 12</td>
<td>NS</td>
</tr>
<tr>
<td>Male</td>
<td>12 (66.6%)</td>
<td>14 (63.6%)</td>
<td>NS</td>
</tr>
<tr>
<td>Female</td>
<td>05 (27.7%)</td>
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</tr>
<tr>
<td>HT</td>
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<tr>
<td>Smoking</td>
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<tr>
<td>Non-alcoholic</td>
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</tr>
<tr>
<td>SLS</td>
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cHSP, *Clamydia pneumoniae* heat shock protein; HT, hypertension; DM, diabetes mellitus; SLS, sedentary lifestyle; NS, non significant.

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 Na3VO4, 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-1α, Gsk3b, and beta actin and further incubated with the monoclonal goat anti-rabbit IgG conjugated with horseradish peroxidase. Subsequently, they were developed using diaminobenamide 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.

Immunoblotting

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].

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

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

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

**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.

**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.
C. pneumoniae signaling in CAD patients

![Image](image-url)

**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
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, TGFβ1, TGFβ2, APOE, EGR1, CTGF, APOB, LDLR, LPA, LPL, PDGFRB, and VEGFA were significantly (p < 0.001) up-regulated while ELN, CSF2, SERPINB2, 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
C. pneumoniae signaling in CAD patients

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-\(\alpha\) and -\(\gamma\) 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 2](image1.png)  
**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.*

![Figure 3](image2.png)  
**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.*
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 histocompatibility complex 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].

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, NFκB, CCL3, CCL4, CXCL3, CXCL4, CCR5, IL1B, CXCL9, CXCR3, CCR2, CD8, and IL-13 is higher in chSP60 positive CAD patients. Moreover, we found that the expression of CCL3, CCL4, CXCL3, CXCL4, and CCR5 is higher in chSP60 positive CAD patients compared to negative CAD patients. Furthermore, we found that the expression of IFN-γ is higher in chSP60 positive CAD patients compared to 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 histocompatibility complex 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 4** Immunoblotting of c-FLIP and PGC1-α in chSP60 positive and negative coronary artery disease patients. chSP, *Clamyydia pneumoniae* heat shock protein; Cp, *Clamyydia pneumoniae.*

**Figure 5** Immunoblotting of Gsk3b in chSP60 positive and negative coronary artery disease patients. chSP, *Clamyydia pneumoniae* heat shock protein; Cp, *Clamyydia pneumoniae.*
CCL23, CCL24, and CCL25 were higher whereas expression of IL-10, IL-10RA, IL1F10, IL1A, and IFNA2 were lower in C. pneumoniae 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 CXC4L1 can induce signaling in activated T lymphocytes, which results in their chemotactic migration and also suggested a role of CXC4L1 in T cell-mediated immunoregulation [51]. It has also been reported that CXC4L1 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 transcription factors such as NF-κB in these cells. Further, Chlamydia 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 C. pneumoniae positive CAD patients showing C. pneumoniae 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.

Acknowledgements

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