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Molecular and Serological Detection of *Chlamydia Pneumoniae* in Karbala City / Iraq

Jassim Hamza K. Al-Masoudi, Prof. Mohammed A. K. Al-Saadi, Asst.Prof .MaysaSalih M.AL-Shukri

Babylon University, College of Medicine, Iraq

Abstract: *This work aims at the genotyping characterization for Chlamydiapneumoniae in patients with cardiovascular diseases in Karbala Province-Iraq.Blood samples were collected from each subject. Bacterial DNA was extracted from patient fresh blood. Samples were subjected for molecular detection of C. pneumoniae by using convential polymerase chain reaction (PCR) depending on 16SrRNA. Also, virulence genes for C.pneumoniae including outer membrane protein (omp1) and heat shock protein (hsp) were detected byPCR technique. Five samples (7.75%) revealed positive results for C. pneumoniae by PCR technique in blood samples of patients group, whereas all control samples were negative. Regarding virulence genes, four samples (80%) out of five samples expressed positive for omp1, whereas only two samples (40%) expressed positive result for hsp.*

Keywords: *Chlamydia pneumonia, PCR*

1. INTRODUCTION

Chlamydia pneumoniae causes upper and lower respiratory tract infections worldwide [1] *Chlamydia* was first believed to be viruses for their obligate intracellular requirements. *Chlamydia* was belong to *Rickettsia* until the genus *Chlamydia* was established [2]The organisms is a species of rod-shaped, Gram-negative bacteria, non- motile and obligate intracellular parasite [3] that is known to be a major cause for respiratory disease and has been linked to cardiovascular disease [4] It is an airborne bacteria, small to viruses, *C. pneumoniae* that is a parasitic organism that cannot be reproduce outside of the host cell and is therefore dependent on the metabolism of host cell for their survival[5].

Heat shock proteins belong to a family of about twenty-four proteins that are essential in regulating the molecular response to the vessel wall below both normal and stressed environments *hsp*sare a class of highly preserved proteins produced by all organisms as a result of stress anddamage. Specifically it has been proposed that the immune response to organisms *hsp60* may lead to autoimmunity to human-*hsp60* and by that means promote the improvement of atherosclerosis [6].Up regulation of *hsps* is mediated by heat shock transcription factors binding to the adjusting elements of the *hsp* gene promoters [7].

The *momp* of *C. pneumoniae* has been described as an immunodominant antigen. Most of the immunogenic epitopes of *momp* seem to be conformation dependent because monoclonal antibodies elevated against denatured*momp* show low binding affinity. It is not known why there are so many polymorphic outer membrane protein *pomp* genes and whether EBs could switch the manifestation of the homologous *omp*s or *pmp*s and thus change their membranes in *C. pneumonia* [8].

The polymerase chain reaction PCR has been used to amplify DNA from *C. pneumoniae*. The PCR is based on primers derived from the major outer membrane protein gene sequence of *Chlamydia trachomatis*, but these primers were not specific for *C. pneumonia*have also defined use of PCR for the detection of *C. trachomatis* and *C. psittaci* by using primers based on the sequences of the *16SrRNA* of those organisms. The reaction of the *16SrRNA* primers with *C. pneumoniae* was not distinguished, and the *16SrRNA* sequence of *C.pneumoniae* was not available for comparison [9]. Despite their superior sensitivity, there are problems in clinical samples both false positive false negative and results can occur. The possible errors can be

attributed to preanalytical procedures, DNA extraction and sample preparation, interpretation and, assay validation assay design, confirmation of results [10].

2. MATERIALS AND METHODS

The DNA extraction from fresh blood specimens were carried out according to the manual of manufacturer of Geneaid Company.

3. DETECTION OF VIRULENCE FACTORS BY PCR

A pair of*hsp* primer was used for the amplification of a fragment gene, a pair of *omp* primers was used for the amplification of fragment genes and a pair of *16SrRNA* primers was used for amplification of fragment genes. A single reaction mixture contained 2.5µl of upstream primers, 2.5µl of downstream primer, 5µl of DNA extraction, 12.5µl of master mix and 2.5µl of nuclease free water, to obtain a total volume 25µl. The resulting PCR products were run in 1.5 % agarose gel. The primers sequences and PCR condition are listed in the (table 1).

Table1. *Primers sequences and PCR condition to detect Chlamydia pneumoniae genes*

Genes	Primer sequence (5□-3□)	Size of product bp	PCR condition	Reference
*omp*1	Sense – TTA TTA ATT GAT GGT ACA ATA- Antisense – ATC TAC GGC AGT AGT ATA GTT-	207	**95ºC 5min 1x** **94ºC 30s** **50ºC 45s 40x** **72ºC 1min** **72ºC 10min 1x**	[11]
Hsp70	Sense -AAGTCGCTAAAGCTCCTACTC Antisense - CTTCATCAAAGTCGTCTCCA	760	**94ºC 5min 1x** **94ºC 1min** **52ºC 50s 30x** **72ºC 1min** **72ºC 10min 1x**	[12]
*C.pn 16S*rRNA	sense TGACAACTGTAGAAATACAGC antisense ATTTATAGGAGAGAGGCG	463	**95ºC 2.5min 1x** **94ºC 30sec** **53ºC 1min 30x** **72ºC 1min** **72ºC 5min 1x**	[13]

4. RESULTS

4.1. Molecular Characterization

The results of gel electrophoresis for DNA bands based on genes specific for *C. pneumoniae16SrRNA* revealed that 5:65 patients (7.7%) were positive for target specific for *C.pneumoniae* gene *16SrRNA* in their blood samples with molecular length 460 base pairs (figure 1).

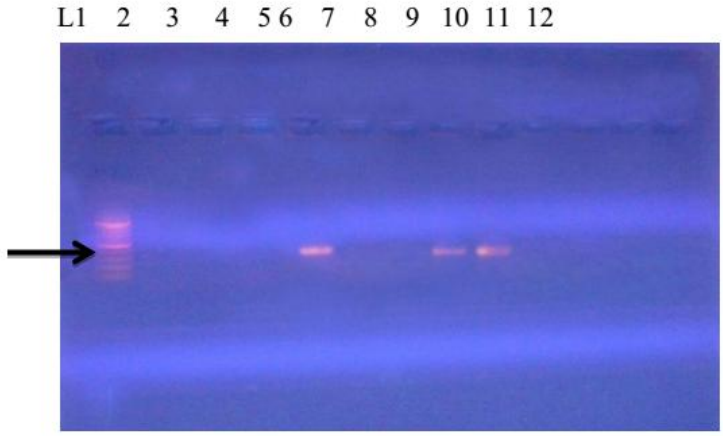

Fig1. *Chlamydia pneumoniae* specific gene *16SrRNA*

This result indicates at first time the presence of *C.pneumoniae* in the blood of patients admitted to the hospitals in Karbala city .Moreover, those patients carrying these bacteria truly due to the fact that PCR technique is considered a golden diagnostic tool [14].

4.2. Outer Membrane Protein (Omp1)

Primers of outer membrane protein*omp1* were used for detecting the presence of *omp1* gene in*C. Pneumoniae* isolates. It has been found that four (80%) isolates of *C. pneumoniae* contain the *omp1* genes with molecular length 207 base pairs. The amplicon was detected in gel electrophoresis and compared with allelic ladder, as shown in (figure 2)**.**

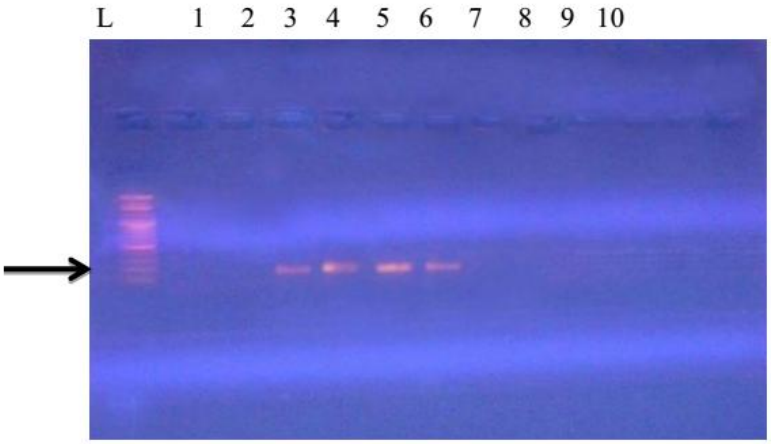

Fig2. *Chlamydia pneumoniae*outer membrane protein*omp*1 gene

4.3. Heat Shock Protein (Hsp)

The analysis of the DNA bands on gel electrophoresis indicated that there are two (40%) isolates of *C. pneumoniae* possess heat shock protein *hsp70* as another virulence factor for this organism with molecular length 766 base pairs (figure 3).

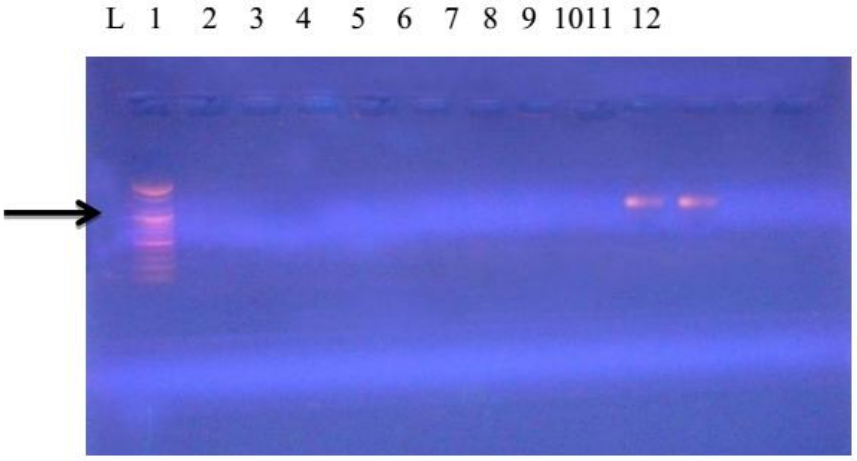

Fig3. *Chlamydia pneumoniaeheat shock protein hspgene*

5. DISCUSSION

The use of *16SrRNA* gene sequences to study bacterial taxonomy and phylogeny has been by far the most communal housekeeping genetic marker used for a number of reasons. the *16SrRNA* gene (1,500 bp) is large enough for informatics purposes .*Chlamydia pneumoniae* played a direct role in the pathogenesis of CVDs mentioned that *C. pneumoniae* was isolated in (23.4%) of CVD patients. *C. pneumoniae* DNA was detected in 14% in patients with CVDs in Canada [15]The result of this work was also supported by other previous researches that have noted this pathogenic bacterium The rate of detection for *C. pneumoniae* depends widely on the sensitivity of diagnostic tool .Several studies have imputed causativeand indirect association roles for this bacterium in the pathogenesis of vascular diseases [17].The cell wall structure of *C. pneumoniae* seems to be much more complicated in its details. The major membrane protein does not carry the species-specific epitopes. Furthermore, it is only feebly immunogenic. The species-specific *C. pneumoniae* epitopes are evidently located on the closely folded polymorphic membrane proteins *pmp1*.The *omps*of obligate intracellular bacteria play a direct role in the process of adaptation by facilitating interactions between the bacterial cell and its host cell. The *omp1* of the *Chlamydial*

elementary body EB must provide components for protection against the environment outside the host, defense against the host immune response, and attachment to host cells..It is not known why there are so many polymorphic *omp*, *pomp* genes *pmp* and whether EBs could switch the expression of the homologous *omps* or *pmps* and thus change their membranes in *C. pneumonia* [8].

The existence of silent genes in this gene family may offer the possibility of varying surface composition. Thus, regulation of the promoter for a surface composition protein gene leads to the expression of the gene. By this mechanism, the expression of the surface composition protein genes can be switched on and off so that the bacteria have the ability to vary both the antigenicity and structure of the surface composition. The *omp* shows similarities to surface composition proteins. Therefore, analysis of gene organization in the genome offers important information for the regulation of *omp* gene expression [17].

Heat shock protein can fall off of the EBs and act as a major adhesion protein, playing an important role in the pathogenesis of *C. Pneumoniae* related diseases including respiratory tract diseases and vascular diseases. During inflammatory process, *hsp* may initiate the secretion of interleukin IL-6, IL-1β, IL-8, and tumor necrosis factor TNF-α in vascular cells, mononuclear cells and dendritic cells [18].

Kol[19] find that bacterial *hsp60*, like another microbial product such as LPS, may activate the innate immune system. The findings of Kol study suggest a new function for *chlamydial* and also human *hsp60* the activation of intrinsic human vascular cells. The *hsp60* markedly increased E-select in and Intercellular adhesion molecule -1 ICAM-1 levels on endothelial cells and Vascular cell adhesion molecule -1VCAM-1 to a much lesser extent. Specific DNA sequences in the heat shock gene promoters when attached to DNA, heat shock factors HSF becomes phosphorylated. The transcriptional activation of the heat shock genes leads to elevated levels of *hsps* and to the formation of heat shock factor, *hsp* complexes. Finally, the trimeric forms of heat shock factor dissociate from the DNA and are converted back into non-active monomers. [20]. The stress dependent conversion of heat shock factor into its active form implies that HSF is negatively regulated. The *hsps* themselves may regulate the heatshock gene expression via an auto regulatory loop. According to this hypothesis, the increased concentrations of misfolded proteins formed during stress bind *hsp70*, resulting in the activation of HSF[21].Heat shock proteins have been divided into sub families, which have been named according to their approximate molecular weight . The five main groups are *hsp100, hsp90, hsp70, hsp60* and the small *hsps*[22].Apart from size, the members of a given family also have several other features in common. They show, for example, a high degree of sequence homology among different species. Their functions are also very much alike regardless of whether bacteria, yeast, plant or animal cells are involved.*hsp70* has been found to be associated with the ribosomal subunits, and it is assumed to stabilize their structure. Ribosomal RNA synthesis, as well as protein synthesis have been shown to be protected by *hsps*. Furthermore, *hsps*have a role in antigen processing and presentation. Especially members of the *hsp70* family are critically involved [22].

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