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ISOLATION OF CHLAMYDIA PNEUMONIAE FROM SERUM SAMPLES OF THE PATIENTS WITH ACUTE CORONARY SYNDROME

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BACKGROUND: Limited body of evidence suggests that lipopolysaccharide of *C. pneumoniae* as well as *C. pneumoniae*-specific immune complexes can be detected and isolated from human serum. The aim of this study was to investigate the presence of viable elementary bodies of *C.pneumoniae* in serum samples of patients with acute coronary syndrome and healthy volunteers.

MATERIAL AND METHODS: Serum specimens from 26 healthy volunteers and 56 patients with acute coronary syndrome were examined subsequently by serological (*C.pneumoniae*-specific IgA and IgG), PCR-based and bacteriological methods. Conventional, nested and TaqMan PCR were used to detect *C.pneumoniae* genetic markers (ompA and 16S rRNA) in DNA from serum specimens extracted with different methods. An alternative protocol which included culturing high-speed serum sediments in HL cells and further *C.pneumoniae* growth evaluation with immunofluorescence analysis and TaqMan PCR was established. Pellet fraction of PCR-positive serum specimens was also examined by immunoelectron microscopy.

RESULTS: Best efficiency of final PCR product recovery from serum specimens has been shown with specific *C. pneumoniae* primers using phenol-chloroform DNA extraction protocol. TaqMan PCR analysis revealed that human serum of patients with acute coronary syndrome may contain genetic markers of *C. pneumoniae* with bacterial load range from 200 to 2000 copies/ml serum. However, reliability and reproducibility of TaqMan PCR were poor for serum specimens with low bacterial copy number (<200 /ml). Combination of bacteriological, immunofluorescence and PCR- based protocols applied for the evaluating HL cells infected with serum sediments revealed that 21.0 % of the patients with acute coronary syndrome have viable forms *C.pneumoniae* in serum. The detection rate of *C.pneumoniae* in healthy volunteers was much lower (7.7%). Immunological profile of the patients did not match accurately *C.pneumoniae* detection rate in serum specimens. Elementary bodies of *C.pneumoniae* with typical ultrastructural characteristics were also identified in serum sediments using immunoelectron microscopy.

Conclusions: Viable forms *C. pneumoniae* with typical electron microscopic structure can be identified and isolated from serum specimens of the patients with acute coronary syndrome and some healthy volunteers. Increased detection rate of *C. pneumoniae* in serum among the patients with an acute coronary syndrome may contribute towards enhanced pro-inflammatory status in cardiovascular patients and development of secondary complications of atherosclerosis.

Key Word :
Chlamydia pneumoniae, PCR, human serum, acute coronary syndrome, cultured cells

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