Is Chlamydia Pneumonia An Independent Risk Factor In Ischemic Heart Disease?

Eman Sh. AL-Obeidy*, Basil. N. Saeed **

ABSTRACT:

BACKGROUND:

Immune system may interplay between Chlamydia pneumoniae infection and ischemic heart disease (IHD).Major histocompatibility genes regulate innate and adaptive immunity..

OBJECTIVE:

This study was established to shed light on the possible association between ischemic heart disease (IHD) with Chlamydia pneumoniae infection and HLA antigens.

PATIENTS AND METHODS:

Microlymphocytotoxicity assay has been applied for HLA-typing of 150 blood samples of 100 IHD patients and 50 healthy normal controls, In addition enzyme linked immunosorbant assay (ELISA) used to detedct C pnuminiae IgA and IgG.

RESULTS:

An increased frequency of HLA-A*2, B*35 alleland HLA-A*2-B*35 haplotype was observed for patients group versus control group with P-value (0.0001, 0.05, and 0.001) respectively.

CONCLUSION:

This finding demonstrated that the HLA-B*35 positive haplotypes confer the C.pneumoniae-related risk for IHD. HLA-DR3,DR4 and DR7 might play a role in AIH susceptibility.

KEY WORDS: ischemic heart disease, HLA, C. pneumoniae.

INTRODUCTION:

Chlamydia pneumoniae is a common cause of respiratory tract infections. Like all chlamydial species, it has a tendency to cause chronic infections. This may lead to severe sequels, such as chronic obstructive pulmonary disease [(1)] and cardiovascular diseases[(2)]. Most likely, all individuals get infected with *C. pneumoniae* during their lifetime, but many of them are capable of resolving the infection, and only some become chronically infected. It is assumed that in order to maintain a persistent infection and to evade host defense mechanisms, chlamydiae have developed specific strategies[(3)], e.g. by being an obligatory intracellular organism, and by having a unique and complicated life-cycle[(4)]. An aberrant and persistent form of *C. pneumoniae* can be induced by IFN-γ[(3)], antibiotics[(5)]and by tobacco smoke[(6)]in vitro cell cultures. In vivo, however, immunogenetic factors[(7)] of the host also contribute to the infection outcome.

Major Histocompatibility Complex (MHC) region takes part in innate and adaptive immunity[(8)]. This molecules present microbial peptides to the appropriate subsets of T-cells. Patients with specific HLA genes are susceptible or resistant to certain viral and non-viral pathogens [(9,10)]. IgA and IgG antibodies against *C. pneumoniae*[(8)] and immune complexes (ICs)[(11,12)] have frequently found to be present in the sera of patients with ischemic heart disease (IHD). In addition to *C. pneumoniae*, an infectious etiology of atherosclerosis and IHD has been implicated with *Helicobacter pylori*, herpes simplex virus and cytomegalovirus[(18)]. Several case control studies suggest that vaccination against influenza may reduce the risk of myocardial infarction[(5,8)]. One of the preferred alleles in the influenza A virus specific cytotoxic T lymphocyte response is HLA-B*3501; preferential HLA usage is dependent on the type of the virus and determines differential cytokine expression[(3, 7)]. The HLA-B*35 allele has also been associated with chronic hepatitis B, hepatitis C, and herpes simplex virus infections, Epstein-Barr virus seropositivity, and with cytotoxic responses against *Aspergillus* and *Mycobacterium tuberculosis* [(10, 12)].

Recently, it has been shown that *C. pneumoniae* infected host cells can induce the genes involved in apoptosis[(6)], while HLA-B35 influences the apoptosis rate [(3)].

Therefore, in this study we assessed in the patients with IHD whether the MHC genes associated with serological markers of *C. pneumoniae* infection:

* Immunology, Medical City.
** Departement of Medicin, Medical City.

elevated specific IgA and IgG antibody levels.

PATIENTS AND METHODS:

PATIENTS:

The present study included 100 Arab, Iraqi IHD patients (25females and100 males), attending department of cardiology; Baghdad Teaching Hospital in a period between December 2008 and June 2009.Their age raged between 39-75 years, compared with 50 healthy individuals (age and sex matched). Those adult patients did not have any of the conventional risk factors for coronary artery disease (i.e. hypertension, diabetes, hypercholesterolanemia, smoking with negative family history.

Laboratory investigation:-

The sera were tested for C. pneumoniae (IgA and IgG) using Enzyme-Linked Immunosorbent Assay (ELISA) technique used human IgG Fc as the antigen coated the microwells plate and isotype-specific horse antibodies coupled to radish peroxidase; result were expressed as the optical density. A level >20 IU/ml and 40 IU/ml were considered positive for IgA and IgG respectively.

PCR technique has Microlymphocytotoxicity assay has been applied for HLA-typing [13].

Statistical analysis was done using SPSS version 7.5 computer software (Statistical Package for Social Sciences).

The mean value with the standard deviation (SD) for each value was determined .P- value less than the 0.05 level of significance was considered statistically significant.

RESULTS:

Table 1: Clinical and dimorphic features of the studying groups

Clinical and dimorphic features	IHD patients (No=100)	Healthy control (N0=50)	P-value
Age (years)	57±12.5	52±10.5	NS
Male	75 (75%)	39(56%)	NS
Hypertension	< | 40 | 90	< | 40 | 90	NS
Diabetes	Fasting<125 mg/dl	Random <140 mg/dl	NS
HLA status:-			
HLA*02 HLA-B*35 HLA-A*02-B*35	37 (37%) 70 (70%) 30 (30 %)	20 (40 %) 12 (24%) 8 (16 %)	0.007 0.001 0.05

*NS=non significant

The above table showed the demographic and clinical picture for the studying groups. It revealed that the majority of patients was male (75%).

Mean age for IHD patients was 57±12.5 which slightly compatible with healthy control group age.

Hypertension, diabetes and hypercholesterolemia have been excluded

Table 2: The difference in mean serum level of C. penumoniae -IgA (IU/ml) between studied groups.

No of IHD patients with Positive C Pneumoniae-IgA	IHD patients N0=(48)	Healthy control group (No.=9)	P-value
Values			
Minimum concentration	29.4	20.9	
Maximum concentration	90.8	38.6	
Median	53.4	26.2	0.01

Table 3: The difference in mean serum level of C. pneumoniae -IgG (IU/ml) between studied groups.

No of IHD patients with Positive C Pneumoniae-IgG	IHD patients (No.=60)	Healthy group (No.=10)	P-value
Values			
Minimum concentration	45.0	42.0	
Maximum concentration	150	65.7	
Median	95.6	53.8	0.001

Table 4: Significant association of MHC genes and haplotypes with the single marker of C. pneumoniae infections (IgA and IgG antibodies) in patients with IHD.

Distribution of MHC genes in IHD patients with positive IgA or IgG C. pneumoniae	IgA	IgG	P-value
Patients with IHD (No.=75)			
-HLA-A*2 Presence (n=37) Absence(n=38)	22 (59.4%)	16 (43.2%)	0.07
-HLA-B*35 Presence (n=60) Absence(n=15)	30 (50%)	40 (66.6%)	0.05
-HLA-A*2-B*35 Presence (n=25) Absence(n=50)	10(40%)	20 (80%)	0.001

DISCUSSION:

This study shows, for the first time, that markers of *C. pneumoniae* infection are associated with HLA-B*35 -related haplotypes in the patients with IHD. Furthermore, among these patients, the prevalence of *C. pneumoniae* infection markers were more pronounced. In the controls, HLA-B*35 was not associated with *C. pneumoniae* infection. HLA-B*35 alone, or together with the other functionally important genes on the haplotype A*02−B*35 may provide one possible link between IHD and *C. pneumoniae* infection.

C. pneumoniae infection has been linked to IHD by several methodological approaches, e.g. by seroepidemiological studies, and by demonstrating the presence of *C. pneumoniae* in atherosclerotic lesions by culture, immunocytochemistry, PCR and electron microscopy. In the present study, we used elevated anti-chlamydial IgA and IgG as markers suggesting chronic *C. pneumoniae* infection.

Thus, antibodies are only indirect evidence of chronic Chlamydia infection; however IgA will not stay high without a continuous infection [10,14].

The prevalence of these markers was significantly higher in the patients than in the controls. Male sex is established risk factors for IHD, and also predispose to *C. pneumoniae* infection[15,16]. Also in this study, male gender, when related to HLAB*35, was definite risk factors for *C. pneumoniae* infection in patients with IHD.

Thus, the suggested chronic *C. pneumoniae* infection in patients with IHD seems to be the result of multiple factors and among them the HLAB*35 positive haplotype may play an important role.

Associations between immunity and genes regulating inflammation with IHDand *C. pneumoniae* infection have been studied only occasionally[9,17]. Dahlén *et al.* studied class II HLA genes and showed that *C. pneumoniae* antibodies associated with HLADRB1*03 and -DRB1*13, but the association was not later confirmed by the same group[4,16]. While our study shown an association between IHD and HLA-B*35 -related haplotypes. The results in this study may indicate that the elevated markers of *C. pneumoniae* infection associate especially with the

presence of HLA-B*35 allele on the haplotype HLAA*03−B*35.

The results of our study may indicate that HLA-B35 presents *C. pneumoniae* peptides, and may further provide an environment that predetermines the establishment of persistent *C. pneumoniae* infection. HLA-B*35 allele has been shown to confer a significant proapoptotic influence to different kind of cells. It may promote pathologic cell activation and proliferation and gene transcription through the control of intracellular cation concentration[16]. *C. pneumoniae* infected monocyte cells are shown to up-regulate many genes including vasoconstrictor endothelin-1[17].

CONCLUSION:

Our results show that the HLA-B*35 positive haplotypes confer the *C. pneumoniae*-related risk for IHD.

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