THE ASSOCIATION OF HLA CLASS II DR ALLELES WITH THE OUTCOME OF HCV INFECTION IN EGYPTIAN CHILDREN AND ADOLESCENTS

Thesis

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بِسْمِ اللّهِ الرحْمَنِ الرحِيمِ

"قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلا مَا عَلمْتَنَا إِنكَ أَنتَ الْعَلِيمُ الْحَكِيم"

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List of abbreviations

AASLD	***American association of the study of liver diseases***
Ab	***Antibody***
AC	***Asymptomatic carriers***
AFP	***Alfa feotoprotein***
AIH	***Autoimmune Hepatitis***
ALT	***Alanine aminotransferase***
APC	***Antigen presenting cell***
Arg	***Arginine***
ATL	***Adult T cell Leukemia***
BMT	***Bone marrow transplantation***
CD	***Celiac Disease***
CD	***Cluster of differentiation***
CD4+	***Helper T cells***
CD8+	***Cytotoxic T cells***
CHC	***Chronic hepatitis C***
CLD	***Chronic liver disease***
CTLS	***Cytolytic T lymphocytes***
CVID	***Common variable immunedeficiency disease***
DCs	***Dendritic cell***
E	***Envelope***
EIA	***Immunosorbant assay***
ETR	***End treatment response***
FMDV	***Foot and mouth disease virus***
GVHD	***Graft versus host disease***
HA	***Hyaluronic acid***
HAART	***Highly active antiretroviral therapy***
HAM	***Human cell Leukemia virus 1- associated myelopathy***
HAV	***Hepatitis A Virus***
HBV	***Hepatitis B Virus***
HCC	***Hepatocellular carcinoma***
HCV	***Hepatitis C Virus***
HEV	***Hepatitis E Virus***
HIV	***Human Immune Deficiency Virus***
HLA	***Human leucocyte Antigen***
HSCT	***Haematopoitic stem cell transplantation***
HVR	***Hypervariable region***
IDDM	***Insulin Dependent Diabetes Mellitus***
IDUS	***Injecting drug users***
IFN	***Interferon***
IG	***Immunoglobulin***
IgM	***Immunoglobulin M***
IM	***Intramascular***
IV	***Intravenous***
JCA	***Juvenile chronic arthritis***
Kd	***Kilo Dalton***
LC	***Liver cirrhosis***
LDALT	***Living donor adult liver transplantation***
LRLT	***Liver related liver transplantation***

Lys	*Lysine*
MHC	*Major Histocompatability Complex*
MLC	*Mixed lymphocyte culture*
MS	*Multiple Sclerosis*
NC	*Normal control*
NHANES	*National Health and Nutrition Examination and Survey*
NS	*Non structural*
Peg	*Pegylated*
R	*Recovered*
RA	*Rheumatoid Arthritis*
RFLP	*Restriction Fragments Length Polymorphism*
RIBA	*Recmbinant immunoblot assay*
RNA	*Ribonucleic acid*
SLE	*Systemic Lupus Erythematosis*
SSO	*Sequence Specific Oligonucleotide Typing*
SSP	*Sequence Specific Priming*
SVR	*Sustained viral response*
T1D	*Type 1 Diabetes*
TAP	*Transporters associated with processing*
TB	*Tuberculosis*
TCR	*T cell receptor*
US	*United States of America*
UTR	*Untranslated regions*
VP	*Viral persistence*
VSV	*Vesicular Stomatitis virus*
WHO	*World Health Organization*

Abstract

Hepatitis C virus (HCV) infection is a global medical problem. The immune response to HCV is an important determinant of disease evolution and can be influenced by various host factors. HLA class II may play an important role in immune response against HCV. The association between HLA class II antigen and HCV in different ethnic populations that has been reported is controversial. Therefore the objective of the present study was to determine the distribution of HLA class II DRB1 alleles, to confirm the influence of these antigens on the outcome of HCV infection and to assess the relationship between these antigens with clinical , laboratory and histological state of the liver among Egyptian children and adolescents with chronic HCV infection . Methods: HLA class II DNA typing was performed by means of Hybridization with sequence specific oligonucleotide probes, after amplification of the second exon of the DRB1 genes using the RELI TM SSO HLA- DRB typing test. Forty six Egyptian patients with chronic HCV infection were included in the study (29 males and 17 females) with age range 3-17 years (10.4 years (y) ±4.232); and 20 normal healthy control subjects. Results: HLA DRB1*15 was found significantly with reduced frequency among our cases when compared with controls (8.7%vs 45%) P<0.01. There were higher frequencies of HLA-DRB1*03, DRB1*04 and DRB1*13 in patients compared with controls (45.6, 39.1 and 26.1%) respectively, indicating a possible implication of these alleles with chronic HCV infection. There was no significant correlation upon comparing the frequency of these alleles with demographic characteristics, risk factors of HCV acquisition, co-morbid condition, clinical presentation, abdominal ultrasonographic picture, biochemical profile and histopathological examination in our patients.

However; patients possessing the allele DRB1*03 were encountered with significant reduced platelet count p=0.03 and this allele was presented with high frequency in patients with minimal grade of inflammation but it did not reach a statistical significance p=0.06, though it was very close. Patients with DRB1*04 had significantly low serum albumin p=0.04 and patients with DRB1*13 had a significant high serum AST levels p=0.05. These findings agree with the association of these alleles and the development of chronic HCV infection.

Therefore, it can be concluded that the allele DRB1*15 is associated with protection from chronic HCV infection and the alleles DRB1*03-*04 -*13 could be associated with chronic HCV infection in Egyptian patients; However larger group of patients is needed for statistical values to be more significant.

Key words: Children; Egypt; HLA class II; Hepatitis C virus; Liver

INTRODUCTION AND AIM OF THE WORK

Hepatitis C virus infection is an increasingly major health problem, threat and concern world wide. There are 170 million infected individuals world wide, i.e., the prevalence of infection is nearly 3% **(Schafer, et al 2004).**

Egypt has higher rates of HCV than neighboring countries as well as other countries in the world with comparable socioeconomic conditions and hygienic standards for invasive medical, dental, or paramedical procedures **(Zakaria, et al 2005).**

Chronic hepatitis C varies greatly in its course and outcome. At one end of the spectrum are patients who have no signs or symptoms of liver disease and completely normal levels of serum liver enzymes. Liver biopsy usually shows some degree of chronic hepatitis, but the degree of injury is usually mild, and the overall prognosis may be good. At the other end of the spectrum are patients with severe hepatitis C who have symptoms, HCV RNA in serum, and elevated serum liver enzymes, and who ultimately develop cirrhosis and end-stage liver disease. In the middle of the spectrum are many patients who have few or no symptoms, mild to moderate elevations in liver enzymes, and an uncertain prognosis

(Alter & Seeff, 2000).

HCV in pediatric patients is in general terms a slow progressive disease, but a number of patients have a more aggressive course leading to early cirrhosis. Therefore, children with chronic HCV need to have early evaluation, consistent follow-up and despite repeated normal or mildly abnormal transaminases, it is advisable to obtain baseline liver histology with potential follow-up

biopsies in 5-10 yr to tailor timing for treatment **(Rumbo, 2005).**

The class I and class II human leukocyte antigens (HLA) are central to the host immune response and thus are ideal candidate genes to investigate for associations with HCV outcomes. Class I and class II HLA are encoded by the most polymorphic genes known and present antigen to CD8+ cytotoxic T cells and CD4+ helper T cells, respectively. Polymorphisms in the peptide binding regions of these molecules determine antigenic specificities and the strength of the immune response to a given pathogen. Certain HLA alleles have been shown to influence the outcome of other chronic viral infections **(Thio et al, 2002)**.

HLA class II antigen appears crucial for resolution or progression of HCV patients. The punctual identification of those genetic factors may, therefore, prove to be useful in predicting disease evolution, in guiding the appropriate therapy for patients with poor prognosis, and in encouraging the development of new therapeutic strategies **(Scotto et al, 2003).**

Patients with chronic hepatitis C and normal ALT levels have less severe liver disease than those with elevated ALT levels. This particular biochemical outcome may be explained, at least in part, by host immunogenetic factors such as presence of HLA DRB1*11**(Renou. et al, 2002).**

In a Chinese study the association between HCV genotypes, HLA DRB alleles and patients response to IFN-alpha and ribavirin therapy displayed complete response to treatment with HLA DRB1*07 and HLA DRB1*04 demonstrated no response, which proves that it

is necessary to adjust the host's immune status to accelerate the clearance of HCV**(Jiao & Wang, 2005).**

Another Study done on Egyptian haemophilic children and adults showed that certain HLA DR alleles as DRB1*0101 and DRB1*0301 may have a role in HCV clearance and persistence **(Hamed et al, 2003).**

Extensive allele diversity is observed in HLA associations with susceptibility and protection regarding HCV infection and disease progression in different global ethnic populations **(Singh et al, 2007).**

AIM OF THE WORK

The aim of this study is to asses the relationship between HLA class II antigens with the clinical, laboratory and histopathological state of the liver among Egyptian children and adolescents with chronic HCV infection.