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Original Article

The association of HLA class II DR B1 alleles with HCV infection in Egyptian children

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ABSTRACT

Background and study aims: Human leucocyte antigens (HLA) class II appear to play an important role in the individual's immune response to viral infection. The aim of this study is to assess the relationship between HLA class II antigens with the clinical, laboratory and histopathological state of the liver in Egyptian children and adolescents with chronic hepatitis C virus (HCV) infection.

Patients and methods: The study included 46 chronically infected HCV children and adolescents without – hepatitis B virus (HBV) nor human immunodeficiency virus – (HIV). Their mean age was 10.4 ± 4.23 years (3–17). HLA-DRB typing was done by polymerase chain reaction (PCR) for the patients and 20 control subjects. Biochemical and haematological parameters were assessed as well as a liver biopsy was taken from the included patients.

Results: The most frequent alleles demonstrated among patients were DRB1∗03, DRB1∗04 and DRB1∗13 (45.6%, 39.1% and 26.1%), respectively. Analysis of DRB1 frequencies between patients and control revealed that DRB1∗15 is significantly reduced among patients when compared with the control group ($p < 0.01$). Patients possessing the allele DRB1∗03 had significantly reduced platelet count ($p = 0.03$), and this allele was presented to a greater extent in patients with minimal grade of inflammation. Patients with DRB1∗04 had significantly low serum albumin ($p = 0.04$) and patients with DRB1∗13 had significantly high serum aspartate aminotransferase (AST) levels ($p = 0.05$).

Conclusion: In Egyptian HCV-infected children, special HLA patterns were found; HLA DRB1∗03 was present in nearly half of the patients, while the frequency of HLA DRB1∗15 was significantly reduced among the cases in comparison to the control subjects.

Introduction

Hepatitis C virus (HCV) infection is gaining increasing attention as a global health crisis. Egypt reports the highest prevalence of HCV worldwide, ranging from 6% to more than 40% according to geographical region and demographic group [1].

HCV in paediatric patients is, in general terms, a slow, progressive disease but, in Egyptian studies, alanine aminotransferase (ALT) levels were persistently elevated in 54.1% of the cases. According to El Raziky et al. and El Hawary et al. hepatic fibrosis was present in 72.1% of the children with HCV infection [2,3].

The immune response and class I and class II human leucocyte antigens (HLA) may be important determinants of disease resolution in relation to HCV. It is not clear why many of these individuals remain asymptomatic, without significant liver damage, while others develop severe liver disease that progresses to cirrhosis and hepatocellular carcinoma, or why only some of those who are treated respond to interferon (IFN) therapy [4].

Genetic association studies have strongly suggested that the HLA class II-restricted response may be important in the context of HCV disease outcome, although the implicated genes differ [5–7]. The timely identification of those genetic factors may, therefore, prove to be useful in predicting disease evolution, guiding the appropriate therapy for patients with poor prognosis and in encouraging the development of new therapeutic strategies [8].

Patients with chronic HCV infection 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 the presence of HLA DRB1∗11 [9]. There is also some evidence that class II genes are involved in the outcome of HCV [10–12].

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

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Patients and methods

Study population

Forty-six children and adolescents with chronic HCV infection were enrolled in this study. They were on regular follow-up in the hepatology outpatient clinics of both Cairo University Pediatric Hospital (CUPH) and Students' Health Insurance Hospital, in the period between January and September 2008. All patients were seropositive for both anti-HCV and HCV RNA. Twenty healthy, age- and sex-matched individuals were included as a control group.

Patients seropositive for hepatitis B surface antigen (HBsAg), hepatitis B core antibody (HBc) or human immunodeficiency virus (HIV) antibodies were excluded as well as those with a history of previous interferon therapy. Patients with coagulopathy or thrombocytopenia to a degree precluding a safe percutaneous liver biopsy were also excluded.

After signing an informed consent by parents all patients were subjected to the following: full medical history, clinical examination, abdominal ultrasound and assessment of liver biochemical profile (total and direct serum bilirubin, aspartate aminotransferase (AST), ALT albumin and prothrombin concentration). ALT levels 1–1.5 times the upper limit of normal (ULN) were considered minimally elevated, and levels >1.5 times ULN were considered moderately elevated.

Viral testing

Patient serum samples were tested for anti-HCV using the AxSYM System HCV version 3.0 (Abbott Diagnostics Division, Germany) and for HCV RNA, by a reverse transcription-polymerase chain reaction (RT-PCR) on sera.

The Sera were also tested for HBsAg using the AxSYM® HBsAg (V2) microparticle enzyme immunoassay (MEIA) for qualitative detection of HBs Ag, HBc, using the AxSYM® CORE and HIV antibody using the AxSYM HIV Ag/Ab Combo.

Histopathological evaluation

All patients underwent a liver biopsy. The histological diagnosis was based on the use of standard criteria [13] and the Knodell histological activity index (HAI) and fibrosis stage [14], using a disposable modified Menghini needle.

HLA class II typing

For patients and the control group, genomic DNA was isolated from peripheral white blood cells using a conventional salting procedure. HLA class II DNA typing was performed by means of hybridisation with sequence-specific oligonucleotide (SSO) probes, after amplification of the second exon of the DRB1 genes, using the RELI™ SSO HLA-DRB typing test. The Dynal RELI™ SSO HLA-DRB test is based on three major processes: PCR target amplification, hybridisation of the amplified products to an array of immobilised SSO probes and detection of the probe-bound amplified product by colour formation.

Statistical analysis

All patients' data were tabulated and processed using Statistical Package for Social Sciences (SPSS) software 10.0 for Windows XP. Qualitative data were expressed in number and percent; they were compared using Chi-square test or Fischer's exact test when appropriate. Quantitative data were expressed in terms of mean and standard deviation. They were compared using Student's *t*-test. In all tests, *p* value less than 0.05 was considered to be significant.

Results

This study included 46 children and adolescents, who were HCV-Ab positive and HCV-PCR positive. Their mean age was 10.4 ± 4.23 years (3–17 years), 29 patients were males (63.04%).

The possible risk factor of HCV acquisition was blood transfusion in 54.3% of the cases, followed by surgical interventions in 47.8%, circumcision for both boys and girls in 45.7% and dental procedures in 36.9% of the cases. Positive family contact with an HCV case was present in 14 patients. Nineteen (41.3%) had a co-morbid condition.

HCV was asymptomatic in 43% of patients, while abdominal pain was present in 41.3%, easy fatigability in 47.8%, jaundice in 6.5%, bleeding tendency in 8.6% and only one patient (2.2%) complained of arthralgia. On clinical examination, 45.6% of the patients had hepatomegaly, 10.8% had splenomegaly and none of them had clinically detectable ascites.

Elevated ALT levels were detected in 35 patients (76.1%), of whom 14 (30.4%) had minimal elevation and 21 (45.6%) had moderate elevation.

Analysis of HLA DRB1 distribution among the HCV patients revealed that HLA DRB1∗03 was the most prevalent allele. Analysis of HLA DRB1 distribution among chronic HCV patients versus the control group revealed that the allele HLA DRB1∗15 was found with reduced frequency among the cases ($p < 0.01$) (Table 1).

In multivariate logistic regression analysis in which acquiring the HCV infection is the dependent factor, the only two alleles that showed significant association were B1∗13 and B1∗15 with odds ratio (OR) 11.9, 0.13 and 95% confidence interval (CI) (0.92–154), (0.02–0.7) ($p = 0.058$ and 0.018), respectively. However, male sex and exposure to parenteral risk factors were independently significantly associated with HCV acquisition in such multivariate analyses of both alleles (Tables 2 and 3).

There was no significant statistical difference in HLA DRB1 allele distribution frequency in patients' groups with different ALT levels (normal, minimally and moderately elevated).

The HAI was minimal (1–4) in 24 cases (53.3%), mild (5–8) in 33.3% and moderate to severe (9–18) in 13.3% of the cases. The fibrosis stage in the studied patients was no fibrosis (F0) in 55.6%, minimal fibrosis (F1) in 31.1% and moderate to extensive (F3–4) in 13.3% of the cases. No significant statistical difference was observed among the studied patients in HLA DRB frequency in relation to the grade of inflammation. However, HLA DRB1∗03 was present in 12 out of 24 patients (50%) with minimal grade of inflammation; yet, it did not reach statistical significance ($p = 0.06$). The frequency of HLA DRB1 distribution in the patients

Table 1
HLA DRB alleles frequency among the 20 control subjects versus 46 HCV patients.

DRB1 alleles	Control $n = 20$		Patients $n = 46$		*p* value
	N	%	*N*	%	
DRB1∗01	1	5	5	10.9	0.66
DRB1∗03	6	30	21	45.6	0.24
DRB1∗04	5	25	18	39.1	0.27
DRB1∗07	4	20	8	17.4	1.0
DRB1∗08	0	0	3	6.5	0.55
DRB1∗10	1	5	4	8.7	1.00
DRB1∗11	3	15	4	8.7	0.43
DRB1∗12	0	0	1	2.2	1.0
DRB1∗13	3	15	12	26.1	0.52
DRB1∗14	1	5	5	10.9	0.66
DRB1∗15	9	45	4	8.7	<0.01

Table 2
Univariate logistic regression analysis in which HCV infection is the dependent factor.

	Univariate			
	OR	*p*	Lower CI	Upper CI
Male sex	0.05	0.026	0.004	0.70
Parenteral risk factor	328	0.001	9.8	1091
B1∗01	134	0.24	0.04	4680
B1∗03	40.3	0.02	1.8	894
B1∗04	9.2	0.17	0.39	214
B1∗07	3.0	0.55	0.08	117
B1∗10	0.86	0.94	0.02	35.4
B1∗11	12.7	0.22	0.23	725
B1∗13	172	0.03	0.16	316
B1∗14	7.1	0.31	0.16	316
B1∗15	1	1	0.03	17.9

Table 3
Multivariate analysis in which the HCV infection is the dependent factor.

	Multivariate			
	OR	*p*	Lower CI	Upper CI
A: For B1∗13 included with male sex and parenteral risk factors				
Male sex	0.1	0.047	0.01	0.57
Risk factor	85.2	<0.01	8.5	858
B1∗13	11.9	0.058	0.92	154
B: For B1∗15 included with male sex and parenteral risk factors				
Male sex	0.14	0.056	0.02	1.1
Risk factor	33.6	<0.01	5.7	194
B1∗15	0.13	0.018	0.02	0.7

groups with different fibrosis stage revealed no statistically significant difference.

We further studied patients having each allele versus those who did not, regarding different study parameters. Platelet count was significantly reduced in patients demonstrating the allele HLA DRB1∗03 ($p = 0.03$). Serum albumin was significantly reduced among patients showing the HLA DRB1∗04 allele ($p = 0.04$). Serum AST levels and total leucocytic count were higher in patients with HLA DRB1∗13 ($p = 0.056$ and 0.04, respectively). Otherwise, there was no significant statistical difference regarding demographic characteristics, clinical presentation and ultrasonographic examination between patients acquiring each allele and patients who did not.

Discussion

In the present study, HLA DRB1∗03, HLA DRB1∗04 and HLA DRB1∗13 (45.6%, 39.1% and 26.1%, respectively), were the most prevalent alleles demonstrated. Singh et al. reported that HLA DRB1∗03 was observed with HCV susceptibility across different populations [15]. The alleles HLA DRB1∗03 and DQB1∗0201 have been associated with persistent infection [16], and they may confer susceptibility to cirrhosis [17].

Comparing the distribution of HLA DRB1 among the cases and the control group revealed a significant difference in the distribution of the allele DRB1∗15, it was less frequent in our cases. This may entail that DRB1∗15 may present HCV epitopes to CD4 cells more efficiently than other alleles. Furthermore, in subjects who do not clear the virus, DRB1∗15 may be also critical in limiting the spread of virus, minimizing, thus, liver injury. Zekri et al. reported that HLA DRB1∗15 was absent in all the HCV-positive cases in their study [18]. In a German study HLA DRB1∗15 (B1∗15011) was found to be more frequent with self-limited HCV infection when compared with patients having chronic hepatitis C [19]. Similarly, in an Irish study, DRB1∗15 was found to be associated with viral clearance and protection from chronic infection [20]. In Taiwan, HLA DRBI∗15 was associated with low viral load [21]. In our study, by using multivariate regression analysis we found that HLA DRB1∗13 and HLA DRB1∗15 were the only two significant independent risk factors for HCV infection in Egyptian children. However, due to a wide CI range, we are not certain about these findings, which could be attributed to the small sample size; but this should raise interest to study these two alleles on large numbers of patients to reach a solid conclusion.

Analysis of HLA DRB1 distribution between the cases with normal and elevated ALT showed no significant difference between any of the demonstrated alleles in this study. However, some studies found a significant difference with the DRB1∗11 allele frequency increasing in patients with normal ALT levels compared to those with elevated ALT levels (43% versus 24%; $p < 0.001$) in chronic HCV-infected French and Italian patients, respectively [9,22].

No significant differences were observed in HLA DRB1 frequencies with different grades of inflammation and stages of fibrosis. However, DRB1∗03 was shown to be more frequent in patients with minimal grade of inflammation, yet, it did not reach a statistical difference. In agreement with our results, Yenigun and Durupinar (2002) reported that there was no correlation between HLA DRB1 and stages of chronic HCV disease [11]. Renou et al. (2002) reported a significant association with the allele DRB1∗11 with no or only mild fibrosis (F0–F1 Metavir index) ($p = 0.03$) [9].

According to the current study, patients with the DRB1∗03 allele had reduced platelet count. The possibility of auto-immune destruction of platelets with active HCV could be an explanation [23], as the HLA DR3 allele has been shown to influence the occurrence of autoantibodies or immunological disorders in chronic hepatitis C [24,25]. Patients with DRB1∗04 allele had a significantly low serum albumin and patients with DRB1∗13 had significantly high serum AST levels and this significance is in agreement with the results of other studies that associated these alleles with liver cirrhosis [26,27] and susceptibility to chronic disease [28].

We are aware with the main limitation of this study, which is the relatively small number of cases because of high cost of the test.

However, we can conclude that Egyptian HCV-infected children demonstrate special HLA patterns; HLA DRB1∗03 was present in nearly half of patients, while HLA DRB1∗15 frequency was significantly reduced among the cases in comparison to control subjects.

Conflicts of interest

The authors declared that there was no conflict of interest.

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