

HLA Alleles in Egyptian HCV Genotype-4 Carriers

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Risk factors affecting asymptomatic HCV carriers as well as intrafamilial HCV transmission are not completely known. We hypothesized that immunological factors related to HLA profiles may affect the intrafamilial transmission. We investigated the possible association between HLA class I & II genes and the presence of HCV infection, as well as their possible role in intrafamilial transmission. One hundred forty five individuals comprising 40 families were recruited from bone marrow transplantation (BMT) unit at the National Cancer Institute, Cairo University. Serologic class I and generic class II MHC alleles were determined. Hepatitis C virus was detected by enzyme immunoassay (EIA) and confirmed by INNO-LIA. Detection of viral RNA was also confirmed by RT-PCR and HCV genotyping was done by immunoblotting (INNO-LiPA) and direct sequencing by TrueGene kit. Out of the 145 serum samples, 33 were positive for HCV antibodies by EIA and confirmed by both immunoblotting techniques and RT-PCR. Twenty-eight of them were eligible for HCV typing. The genotypes detected were 4, 2a, 1a and 1b. A mixed infection by more than one genotype was detected in 9 cases. Six families had 2 or more members reactive for HCV antibodies. Intrafamilial transmission was confirmed by HCV-sequence in 3 families. HLA class I & II alleles A28, A29, B14 & DR7 were significantly encountered in HCV positive than negative cases ($p=0.040, 0.003, 0.001$ & 0.010 respectively). In addition, two cases showed evidence of viral clearance, both expressed B50 (21) allele. We conclude that, there is a possible impact of both class I and class II alleles on HCV infection, resistance, clearance and intrafamilial transmission.

Several studies clearly indicated that HCV infection is an important public health problem in Egypt as it shows very high prevalence rates compared to that reported in other countries (Hibbs et al., 1993 and Kamel et al., 1998). Although the transmission of HCV by direct percutaneous exposure to blood and its products is well documented, the virus also causes most cases of community-acquired hepatitis not associated with blood transfusion (Kumar et al., 1998). In general, well-recognized risk groups including blood recipients, parental drug users and hemodialysis patients constitute only 50-60 % of the cases. The remaining part includes cases of sporadic or community-acquired disease (Kumar et al., 1998). In most cases of community acquired HCV infection it is difficult to establish the source of infection (Coltorti et al., 1995)

although person to person transmission, by sexual contact or household contact including intra-familial and vertical transmission, has been implicated (Kumar et al., 1998). Thus the inadequate information available regarding routes of transmission in community acquired HCV infection stresses the importance of understanding the host factors favoring disease susceptibility.

In hepatitis C, susceptibility to infection, the course of disease and even the response to interferon therapy (Almarri et al., 1998) may depend on both viral factors and host's immune response (Asti et al., 1999). Recent studies have suggested that the course of the disease may be influenced by immunogenetic background of the host (Zavaglia, 1998). HLA-DR-11, in particular has been associated with less advanced HCV-related liver disease (Zavaglia, 1998). In addition, it has been

shown in some studies that the expression of HLA-DR2 is significantly associated with a beneficial response to IFN- α therapy independent of an existing cirrhosis (Almarri et al., 1998). Moreover, Kirk et al. (1996) demonstrated that DR-3 allele is usually associated with a significant risk of developing clinical hepatitis in blood recipients. In their study 80% of the DR3 positive cases progressed to hepatitis compared to 42% of DR3 negative recipients. Although the majority of the previous studies had reported a role for class II MHC, yet the role of class I MHC cannot be ignored especially that the immune response to viral infection is mainly effected by CD8+ cells which recognize viral antigens in context of class I MHC (Diepolder et al., 1997). In addition, a CD8 dominated immune status represented by a reversed CD4/CD8 ratio has been claimed to be associated with a better response to interferon treatment. Moreover, we had reported in a previous study that the pattern of recognition of HCV core and nonstructural antigens by antibodies in Egyptian cancer patients and blood donors is different from that reported in other countries. This might reflect a different host-viral interaction (Attia et al., 1996). In this study we extended our work to investigate the correlation between pattern and genotype of HCV on one side and HLA types (class I and class II) of infected patients on the other side. We studied 145 persons represents 40 families, they were subjected to tissue typing in our BMT unit, aiming to: 1-study the possible association between HLA class I, II and HCV infection and 2- to figure out the possible role of MHC in the transmission of HCV infection within family members.

Patients and Methods

Subjects in this study were participants in another study for allogeneic bone marrow transplantation (BMT) at National Cancer Institute, Cairo University, in the year 1995-1996. One hundred and forty individuals were studied comprising 40 families. They were collected

from the bone marrow transplantation (BMT) unit at the National Cancer Institute. They were 23 males and 17 females. Their ages ranged from 20 to 45 years. Family members constituted 105 first degree relatives of the 40 patients (with a minimum of one and a maximum of 6 each family member); all were HLA typed to choose a suitable BMT donor. All had normal abdominal sonography and no evidence of liver disease, all had normal liver and kidney functions. A written approval consent were obtained from each patient included in this study as well as the ethical committee of the NCI were approved the protocol according to the ethical guidelines of the 1975 Declaration of Helsinki.

HLA-Typing

Mononuclear cells were separated from a heparinized blood sample for each subject to detect the HLA antigens. Class I -HLA antigens were determined by standard micro-lymphocytotoxicity assay using 120 ABC Biotest plates (Dreieich-Germany). Generic DR and DQ typing was performed using Biotest kit (Dreieich- Germany). Serum samples were collected and stored at -80°C for subsequent HCV analysis (Terasaki, 1978).

Anti-HCV EIA

ALL samples were tested using commercially available EIA Abbott Diagnostics, USA.

Anti- HCV immunoblot assay

Anti-HCV EIA positive samples were tested by a synthetic immunoblot assay (LIA, Organon Teknika,) as described previously (Attia et al., 1996).

RNA extraction

HCV RNA was extracted from the sera as previously described (Boom et al., 1990).

Oligonucleotide Primers

RT and PCR were performed with a primer pair selected from the highly conserved 5-UTR of HCV genome (Choo et al., 1989). The following sequences were used as antisense primers for c-DNA synthesis HCV-6 {5 -ACC-TCC nucleotides (NT) 319-324}. The internal primers were RB6A and RB6B for amplification of 266 bp of the 5-UTR, RB6A [5 -GTG AGG AAC TAC TGT CTT CAC G-3 (NT 47-68)] and RB6B [5 -ACT CGC AAG CAC CCT ATC AGG (nt. 292-312)].

RT-PCR of HCV

Reverse transcription was performed in a 25 μl reaction volume containing the following: 20 U of RNase inhibitor (Promega Biotec Madison. Wis.), 67 mM Tris. HCl (pH8.8), 17 mM ammonium sulfate, 1 mM

B-mercaptoethanol, 6 μ M EDTA (pH8.0), 0.2 mg/ml of bovine serum albumin (Boehringer), 6 mM Mg Cl₂, 25 ng of primer HCV-6, 0.6 μ l of 25 mM (each) deoxynucleotide triphosphates, 11.5 μ l of the nucleic acid elute and 200 U superscript-II RNase H reverse transcriptase (GIBCO-BRL, Gaithersburg, Md.). The mixture was incubated at room temperature for 5 min and then at 42°C for 60 min. RT product was denatured by incubation for 5 min at 95°C. The PCR was performed in a 50 μ l volume containing the following: 2.5 U Taq polymerase (Perkin- Elmer Cetus), 50 mM Tris. HCl (pH 8.3), 20 mM KCl, 1.2 mM MgCl₂, 1 mg/ml BSA, 12.5 μ l of the RT reaction mixture, 200 u M (each) deoxynucleotide triphosphate, and 100 ng each of primers RB-6A and RB-6B. The samples were denatured at 95°C for 5 min and were subjected to 35 rounds of thermal cycling in a DNA thermal cycler (type 480: Perkin-Elmer Cetus). Each cycle consisted of denaturation for 1 min at 95°C, annealing for 1 min at 55°C, and extension for 2 min at 72°C. After the cycling program, the samples were incubated for 10 min at 72°C. All samples were analyzed twice for HCV RNA by the RT-PCR on different days with identical results. Upon completion of the amplification reaction, 10ul of each PCR reaction product was analyzed by electrophoresis through a 1.2 % agarose gel in Tris-Acetate-EDTA buffer (pH 8.0) and ethidium bromide staining. DNA was transferred from the gel onto nitrocellulose filter using alkaline buffer (4N NaOH). The transferred DNA was cross linked by incubation for 2-3hr at 80°C and the blot was then hybridized with an internal probe (Zekri et al., 1995).

HCV INNO-LiPA genotyping

The line probe assay was used to assess HCV genotypes as previously described (Zekri et al., 2000). Briefly, the 5'-UTR region was amplified using nested PCR with biotinylated primers. The labeled amplicon was allowed to hybridize and mounted on a strip. After stringent washing, streptavidin labeled with alkaline phosphatase was used to trace the hybridized products and nitroblue tetrazolium and 5-bromo-4-chloro-3-indoyl-phosphate were used as a substrate according to the manufacturer's instructions.

HCV genotyping by sequencing

This was done using TRUGENE HCV 5' NC genotyping kit, Visible Genetics Inc. (USA). This kit was used in conjunction with the OpenGene DNA sequencing system. Briefly, sequencing products from the 5'NC region are generated from previously amplified product of the same region by CLIP sequencing. CLIP sequencing allows both directions of the target amplicon to be sequenced simultaneously in the same tube using two different dye-labeled primers

(Cy5 and Cy5.5) for each reaction. The resulting CLIP sequencing segments are then detected using the OpenGene DNA sequencing system. The forward and reverse sequences are combined to form a query sequence. The query sequence in turn compared to the previously characterized isolates in the TRUGENE HCV 5' NC Module of the OpenGene software system in order to determine the HCV genotype of the sample.

Statistical analysis

The HLA class I and II alleles of HCV positive cases were compared to those of HCV negative ones using Fisher's exact test. The HLA alleles of HCV positive patients were compared with their family member types against HCV transmission by the Mcniemans test. Finally, HCV immunoblotting patterns and genotype distribution were compared using Fisher's exact test (Suanders, 1994).

Results

Thirty-three out of the 145 tested sera were positive for the HCV antibodies by EIA. All positive cases were further confirmed to have HCV antibodies using the immunoblotting technique. Thirty-one cases had immunoblotting reactivity pattern E, and 2 cases were of the pattern C1C2 (table-1).

Out of the 40 families studied, 6 had 2 or more of their members reactive for HCV antibodies (Table-2). We compared HLA class I A, B and C in the 33 HCV positive and the 112 HCV negative. HLA-A28, HLA-A29, HLA-B14 and HLA-B63 (15) were significantly encountered in HCV positive than negative cases ($p=0.040, 0.003, 0.001, 0.06$). HLA class II was only performed in 17 HCV positive and 20 HCV negative cases, HLA-DQ1 and HLA-DQ3 were significantly encountered in HCV positive than negative cases ($p= 0.03$) On the other hand, there was a group of alleles which showed higher frequency in HCV negative cases but did not reach statistical significance though it was very close {HLA-A31, HLA-B50 (21), HLA-B41, HLAB-44 (12), HLA-DR11 and HLA-DR15 (2)}, ($P= 0.09, 0.08, 0.08, 0.09, 0.07, 0.07$) (table-3).

Of the 33 HCV positive cases, 28 were eligible for HCV genotyping. The genotypes

detected were 4, 2a, 1a, and 1b in 15, 12, 9 and 1 cases respectively (table-1). A mixed infection by more than one genotype were detected in 9 cases, whereas in 19 cases a single type only was found. The 2 cases showing pattern C1C2 were classified as genotype 1a as well as 3/29 cases of the pattern E. Of the six families that have more than one HCV infected member, 3 families showed similar genotypes (table-3). The 3 HCV infected members in family 1 and 6 expressed B14 while the no affected one (in family-1) expressed B21. In family 2, the affected cases expressed B63. In family 3, the

non-affected member expressed B41. The affected cases in families 4 and 5 expressed B44, A28 and A29 respectively.

One member in each of families 4 and 5 showed positive antibodies even though the virus was not detected by RT-PCR, both expressed B50(21) allele. This allele was also expressed in an affected member in family 6 who expressed B14 as well. In family 4, the only affected member was the father and he expressed B44. All the negative members, mother and siblings expressed B50(21) as well as B44 in 2 of the siblings.

Table 1. HCV immunoblotting pattern and genotype in 33 HCV antibody ELISA reactive cases.

Case no/Family no	* IB Pattern	HCV genotype
1/1	E	4a
2/1	E	4a
3/1	E	4a
4/2	E	2a
5/2	E	2a+1b
6/3	E	4a+1
7/3	E	4a
8/4	E	2a
9/4	E	Neg
10/5	E	2a+4a
11/5	E	Neg
12/6	E	2a
13/6	E	4a
14/6	E	1a
15/7	ND	1a+4b
16/8	E	2a+4a
17/9	E	Neg
18/10	E	2a+4a
19/11	E	Neg
20/12	E	4a
21/13	C1C2	1a
22/14	E	1a
23/15	E	2a+4a
24/16	E	2a
25/17	E	Neg
26/18	E	2a
27/19	E	1a+2a
28/20	E	4a
29/21	ND	1a
30/22	E	2a+4a
31/23	C1C2	1a
32/24	E	1a
33/25	E	4a

* IB= Immunoblotting pattern according to Attia et al. (1996)

Table 2. HCV genotype and HLA of families showing more than one member positive for HCV.

Family No.	IB Pattern	HCV Type	HLA-Type
1	E	4a	A9,30;B14,B38(16);DR4,10;DQ1,3
	E	4a	A1,23(9); B7,14;
	E	4a	A1,-B14,-
	Neg	Neg	A9,30;B38(16);21;DR4,10;DQ1,3
2	E	2a	A2,24(9);B63(15),39(16);DR4,12(5);DQ,3
	E	2a+1b	A2,24(9);B63(15),39(16);DR4,12(5);DQ,3
	Neg	Neg	A23(9),24(9);B39(16),70
3	E	4a+1	A2,33;B75(15),-
	E	4a	A2,33;B75(15),-
	Neg	Neg	A30,26(10);B35,B41
4**	E	2 a	A2,32;B7,44(12);
	E	Neg	A2,-;B35,B50(21);
	Neg	Neg	A2,-;B44(12),50(21);DR15(2),4;DQ1,4
	Neg	Neg	A2,-;B44(12),50(21);DR15(2),4;DQ1,4
	Neg	Neg	A2,32;B35,50(21)
	Neg	Neg	A2,32;B7,50(21)
5	E	2a+4a	A28, A29; B37,-
	E	Neg	A24 (9), 69(28); B37, 50(21);
	Neg	Neg	A3, A29; B37,-
6	E	2a	A3,- ;B14,27;DR1,7,;;DQ,1,2
	E	4a	A3,- ;B14,27;DR1,7,;;DQ,1,2
	E	1a	A2, A3; B14, B50 (21)

* IB= Immunoblotting pattern according to Attia et al. (1996), Neg = Negative

** All cases were siblings except family 4, the 2 HCV positive cases are a husband and a wife.

Table 3. Distribution HLA-class I and II alleles among HCV positive and negative cases

HLA-type	HCV positive no. (%) (33)	HCV Negative no. (%) (112)	p-value
A28	4 (12.0)	4 (3.5)	0.040
A29	5 (15.2)	3 (2.6)	0.003
A31	0 (0.0)	10 (8.1)	0.090
B44(12)	2 (6.1)	22 (19.6)	0.090
B14	9 (27.3)	7 (6.2)	0.001
B63(15)	3 (9.1)	2 (1.8)	0.060
B50(21)	0 (0.0)	10 (8.9)	0.080
B41	1 (3.0)	17 (15.1)	0.080
DR 7*	5 (29.4)	2 (10)	0.010
DR 11*	3 (17.6)	9 (45)	0.070
DR 15(2)*	0 (0.0)	4 (20)	0.070
DQ 1*	9 (52.9)	17 (85)	0.030
DQ 3*	6 (35.2)	14 (70)	0.030

P-values less than 0.05 are considered significant.

* HLA class II was performed for 17 of the HCV positive and 20 HCV negative cases.

Discussion

It has been previously reported that HCV infection is a common and important health problem in Egypt (Hibbs et al., 1993 and Kamel et al., 1998). The 12.5% incidence in this study reflects this general situation of the HCV infection in Egyptian population. In agreement with our results, other studies recorded 12 to 24 % prevalence rates of HCV in the general population of Egypt (El-Ahmady et al., 1994). HCV infection is rarely eradicated and persistence of HCV is the rule with high chronicity rate (Koziel, 1997). Over the past few years there have been several reports about the relation between HLA and the course of hepatitis C. A better understanding of the role of HLA-genetic factors may provide an additional explanation of the variable prevalence of HCV in different communities. Though chronic HCV infection is characterized by polyclonal, multispecific, class I restricted peripheral cytotoxic T-lymphocyte response (Diepolder et al., 1999). Yet reports have consistently shown no association between the course of disease and MHC class I alleles but rather an impact of certain class of MHC II alleles (Ossi et al., 1995, Tibbs et al., 1996 and Jurado et al., 1997).

Several studies have reported MHC-class II restricted CD4+T-cell proliferative responses to several structural and non-structural HCV recombinant proteins in a significant proportion of patients with acute and chronic HCV infection. Such CD4+T-cell response was usually associated with a more benign or self-limited course of infection (Diepolder et al., 1997).

In our study, however, some MHC alleles (both class I and II) were more frequently encountered in HCV positive cases namely HLA-A28, HLA-A29, HLA-B14, HLA-B63(15) and HLA-DR7 and this association was statistically significant. On the other hand, there was a group of alleles which

showed higher frequency in HCV negative cases but did not reach statistical significance though it was very close {HLA-A31, HLA-B50(21), HLA-B41, HLAB-44(12), HLA-DR11 and HLA-DR15(2)}, HLA-DQ1 and 3 were statistically significantly higher in HCV negative individuals.

In agreement with our results, some studies were able to show a strict relationship between HLA alleles and susceptibility to HCV infection. A highly significant relation between HCV susceptibility and DR7 antigen was reported by Jurado et al., 1997, who analyzed HLA class I and II in HCV negative and positive autoimmune hepatitis. They found high correlation between HCV positivity and DR7 antigen ($p=0.008$) but not with class I or other class II alleles in either group of patients. Moreover, DR7 was found to be expressed in a group of chronic active hepatitis patients only when compared to controls, suggesting that it may influence the specific liver involvement in HCV infected patient's (Ossi et al., 1995). On the other hand, a significant increase of HLA-class II DR2 subtype (DRB1*1601, DQB1*0502) was observed in a group of 30 HCV negative patients who despite being exposed for 10.3 ± 2.2 years to a regular blood transfusion program did not show any evidence of HCV infection (Congia et al., 1996). Similarly, HLA-DR15 (B1*15011) was significantly higher in patients with self-limited HCV infection as assessed by the presence of HCV antibodies and absence of HCV-RNA when compared with patients having chronic hepatitis C (Lechmann et al., 1999). In this work, we didn't encounter any DR16 (2); however, this could be attributed to our small sample size or to the genetic make up of the studied members. The other split of DR2 namely DR15 was absent in all HCV positive cases tested and present in 20% of the negative cases. In the study of Barret et al. (1999) on 157 females who were infected

with HCV-contaminated anti-D-immunoglobulin; the DRB1*01 allele was found to be significantly higher in individuals with viral clearance compared to those with chronic infection (27.4% versus 7.1%).

In agreement with our results, regarding the HLA DQ alleles, Tibbs and his colleagues (1996), found that DQA1*03 and DQB1*0302 were present at a significantly lower frequency in patients with chronic HCV infection $p=0.03$ and 0.04 respectively. Similarly, Barret et al. (1999) mentioned in their study that, the HLA haplotype DRB1*1104/ DQB1*0301 seems to contribute to spontaneous clearance of HCV infection. This haplotype was found to be less frequently present in persistent HCV viremic patients $p=0.009$. In a larger study, recruiting patients from 8 different European centers, self limiting HCV infection was associated with HLA DRB1*1101 and DQB1*0301; while persistent HCV infection was associated with HLA DRB1*0701 and DRB4*0101 (Thursz et al., 1999).

On the other hand, few studies concluded that there is no evidence of association between the HLA antigens and HCV infection (Vendon et al., 1994 and Chen et al., 1996). Verdon and his co-workers (1994) did not find any role of a particular HLA antigen in the development of chronic viral hepatitis in hemodialysis patients. In another study, it was found that neither susceptibility to HCV infection nor the biochemical activity of hepatitis and HCV-RNA positivity seem to be related to HLA alleles in Caucasian patients with end-stage renal disease (Chen et al., 1996).

Molecular epidemiologic techniques are widely used nowadays to trace infectious sources and they have become an increasingly important tool of HCV genotyping (Kumar, 1998). In this study genotyping was performed by INNO-LiPA and sequencing on 28 out of the 33 HCV positive cases. Discernible patterns of genotype distribution

have been found in different geographic areas. Worldwide, genotypes 1, 2 & 3 show a broad geographical distribution whereas others are mostly confined to specific geographical regions (Esteban et al., 1995). In Europe and United States, HCV-1a, -1b, -2a, -2b and 3a are the commonest subtypes, whereas in Japan subtypes 1a and 3a virtually do not exist and genotype 1b seems to be the major pathogen in southeast Europe (Lau et al., 1996). Type 4 genotype is prevalent in patients from Northern Africa and the Middle East (Esteban et al., 1995). In our study, type 4 was the most predominant type being encountered in 45.4 % of the patients, which is in agreement with our previous report in which we demonstrated that 57% of HCV infected patients in Egypt, harbor genotype 4. Also, types -1a and -2a were well represented constituting 12.4% and 11.2% respectively (Zekri et al., 2000).

Mixed infection by more than one HCV genotype was found in 9 of the 28 studied cases, which accounted for about one third of the HCV positive cases. On the contrary, mixed infection was reported in 2% and 3.7 % only of European and USA population (Lau et al., 1996 and Maggi et al., 1997). The difference in incidence rates may be contributed to the repeated exposure to HCV infection in the Egyptian population and indicates that infection with one subtype does not provide protection against re-infection with other subtypes. In cases with mixed infection, subtype-4a was the commonest partner being detected in 7 out of the 9 patients. This finding is in concordance with our recently published data of Egyptian patients harboring mixed infection (Zekri et al., 2000).

In this work, 6 families had more than one member with HCV positive antibodies. It is documented that genotyping is a more accurate marker for intra-familial HCV transmission than the simple HCV-RNA positivity; a similar HCV genotype was detected in 3 out of the 6 families. Intra-

familial clustering of HCV infection has been documented especially in areas of intermediate endemicity (Esteban et al., 1995). It is well established that parental route is the commonest mechanism of HCV viral transmission (Kumar et al., 1998), and infection through body fluids of an infected family member cannot be considered a risk factor, since body fluids do not have detectable HCV-RNA by PCR (Hsu et al., 1991). However, previous studies showed a high risk of HCV infection among the household contacts of anti-HCV reactive subjects, which is higher with longer duration of contact with the index case (Coltori et al., 1995 and Kumar et al., 1998).

No attempt has been made to look for the HCV risk factors within these families, which hindered drawing a solid conclusion of the mechanism of viral spread among their members. A study of these factors needed to be done. The three families were infected with types 4a and 2a which are the commonest strains in the study. This might argue against the possible intrafamilial spread. To overcome this problem, genotyping was done by both INNO-LiPA and sequencing which strongly support intrafamilial transmission. Moreover, a much larger sample size of families of infected patients are needed.

Sexual transmission was found to play a minimal role in the transmission of the HCV (Meisel et al., 1995) as no HCV infection was found among husbands of chronically infected women after 15 years of sexual contact. Only one couple of suspected intra-spousal transmission was found in the present study (family 4). However, sexual transmission cannot be documented as the wife showed viral clearance and hence, genotyping could not be done. Another case of viral clearance was documented in this work in family 5. Both cases who cleared the infection, expressed B50(21), a finding, which may suggest that certain HLA alleles, could be associated with viral clearance. The presence

of B50(21) in an HCV positive case in family number 6 was apparently counter balanced by the presence of B*14, an allele reported in our work to be highly associated with HCV positive cases. A comparable situation is also exemplified in family 4 where the positive case expressed B44, an allele showing significant association with negative cases in this study. All the negative cases in this family expressed B50(21), either alone or with B44. B50(21) was not encountered in any of the positive cases in this work, a finding which might suggest that certain alleles would have a stronger impact than others on susceptibility to HCV infection. Also, it seems that, in addition to several other factors, the HLA genetic make up as a whole, not just one allele, determines the outcome of HCV infection.

However, HLA specificities show variation within populations and differ in geographical areas, suggesting that they could be prevalent in response to certain pathogens (Krausa et al., 1995). Therefore, it is not expected to find comparable results in the context of HLA types in relation to HCV in different populations.

We conclude that there is an a possible association between MHC and HCV acquisition, as some of the HLA-antigens seem to be predisposing to contracting community acquired HCV infection, while others might have a protective influence against HCV infection. Analysis of previous studies in the literature as well as the present work show an association between certain HLA alleles with the development of HCV infection (Kirk et al., 1996 and Jurado et al., 1997), with its clearance (Lechmann et al., 1999 and Barret et al., 1999), with the development of chronicity (Thursz et al., 1999), or response to interferon therapy (Almarri et al., 1998). Also, different viral genotypes were reported to be prevalent in different geographical areas (Esteban et al., 1995), which may reflect a different nature of

the pathogen. It has to be taken into consideration that the interaction of the immune system with pathogens could vary from one place to another due to differences related to the pathogen, to the immune system, to genetic make up and/or to environmental factors. Thus, studies involving evaluation of the risk factors related to a particular disease or pathogen have to be performed at a local level. The findings of this study could help in better understanding the pathogenesis of vertical HCV infection and in better identifying the cases at risk. Accordingly, it's of utmost importance that further studies relating both HLA alleles and HCV infection patterns and genotypes be considered on the Egyptian population to detect their impact on the outcome of HCV infection, whether resistance, clearance of infection, development of chronicity or evolution to hepatocellular carcinoma.

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