

Study of the role of HLA and KIR genotypes on the outcome of HCV infection in a sample of Egyptian HCV infected persons

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Abstract: Introduction: The killer cell immunoglobulin-like receptors (KIR) control the activation or inhibition of Natural killer (NK) cells by recognition of specific HLA class I ligands, such that target cell cytolysis occur only when activating signals overcome inhibitory signals. The aim of the study is to demonstrate the effect of HLA class I and KIR gene interaction on the outcome of HCV infection in Egypt. Material and methods: The study included 137 individuals with resolved HCV infection; 17 cases spontaneous resolution and 120 cases with sustained virologic response (SVR) and 125 individuals with chronic HCV infection. Individuals with resolved and others with chronic HCV infection are subjected to genotyping for (HLA class I), (KIR) gene of killer (NK) cells. Results : (KIR2DL3-HLA-C1) was higher in the group with resolved infection (19.7%) relative to that with persistent infection (7.2%) P-value=0.003, whereas the inhibitory receptor ligand (KIR2DL1-HLA-C2) was higher in the group with persistent infection (19.2%) compared to that with resolved infection (9.4%) P-value =0.02. No significant difference was found between the two groups with the following inhibitory KIR-HLA combinations : KIR2DL2-HLA-C1, KIR2DL2/KIR2DL3-HLA-C1 , KIR2DL3-HLA-C1/C2. The activating receptor ligand KIR2DS2-HLA-C1, KIR2DS1-HLA-C2 and KIR3DS1-HLA-Bw4 were not associated with HCV resolution. Conclusion: HLA/KIR genotype plays a role in determining the outcome of HCV infection. In our population, viral resolution was associated with KIR2DL3/HLA-C1 homozygosity, while virus persistence was associated with KIR2DL1/HLA-C2/C2.

Key words: HCV, HLA class I, KIR genes.

INTRODUCTION

Hepatitis C virus infection (HCV) is a major health problem in Egypt, with a high rate of transmission. In addition to viral factors and environmental/behavioral factors (e.g. co-infections or excessive alcohol intake), host genetic factors are believed to exert an impact on the outcome of HCV infection. Interplay of human leukocyte antigen (HLA) restricted T lymphocytes, antibody-secreting B-lymphocytes, NK cells and cytokines, conditions the immune response to viral infections. Effective presentation of viral antigens to CD4+ T cells and CD8+ T cells by HLA class II and class I molecules respectively, is the key regulation of optimum immune response against viral infection, and further dictates viral clearance or persistence (Singh *et al.*, 2007).

The killer cell immunoglobulin-like receptors (KIR) are a set of polymorphic variants with their primary locus on chromosome 19 and control the activation or inhibition of NK-cell response by recognition of specific HLA-class-I ligands. NK-cell mediated target cell cytolysis occurs when inhibitory signals are overcome by activating signals. The inhibitory KIR genes include 2DL1, 2DL2, 2DL3, 3DL1. An allelic dimorphism in amino-acid residues 77 and 80 determines the specificity of KIRs for HLA-C allotypes. KIR2DL1 recognize group 2 HLA-C molecules (HLA-C2) having asparagine at position 77 and lysine at position 80 (Cwn02,0307, 0310, 0315, 04, 05, 06, 0707, 0709, 1204, 1205, 15,1602, 17, 18), whereas KIR2DL2/2DL3 bind group 1 HLA-C molecules (HLA-C1), which include serine 77 and asparagines 80 (Cwn01, 03, 07, 08, 12, 13, 14, 1507, 16)(Colonna *et al.*, 1993; Moretta *et al.*, 1993). It has been suggested that the activating KIRs (2DS1, 2DS2 and 3DS1) recognize the same HLA class I ligands as their inhibitory counterparts, though with a lower affinity, because of the similarity in the extracellular domain (Verheyden *et al.*, 2005). Not only does every HLA-C allotype interact with a KIR, but there also exists KIRs that interact with certain HLA-A and -B alleles (KIR3DL1 for HLA-Bw4 and KIR3DL2 for HLA-A3/11). (Dohring *et al.*, 1996, Gumperz *et al.*, 1995).

The aim of the study is to assess the role of HLA/KIR genes interaction on the outcome of HCV infection in Egypt.

Methodology:

Subjects:

The participants were recruited from several liver centers in Cairo and Giza (Egypt). Many of these patients have received their interferon treatment in Liver Institute of Cairo University. Two groups of cases were participated in this study according to standard criteria:

1-One hundred and twenty five chronic HCV patients (non-responder to Interferon therapy).

2- One hundred and thirty seven resolved HCV cases, they were divided into 2 types :

a. Sustained virological response (SVR) (120 cases). The patient had a negative HCV-RNA after 6 months of completing 48 weeks (nearly one year) therapy. Patients were treated by either Pegylated or short –acting Interferon along with ribavirin (Formann *et al.*, 2006)

b. Spontaneous HCV Resolution (Self-limiting HCV infection) (17 cases). The patient had at least 3 negative HCV-RNA PCR ,6 months apart with positive HCV-Ab done by ELISA on many occasions (Spada *et al.*, 2004).

Exclusion Criteria:

All the cases with HIV, HBV, and shistosoma were excluded from the start of the study.

All chronic HCV participants with immune hepatitis were excluded.

Questionnaire and Clinical Examination:

A written well informed consent was taken from all the participants. All participants were subjected to full history taking, clinical examination and abdominal ultrasonography. All the cases with history of bilharzial infection and treatment, previous surgery and blood transfusion were excluded. The state of HCV infection and the status of the liver and spleen were evaluated by ultrasonography. Type of treatment, its duration and response were recorded.

The study was approved by the Ethical Committee of the National Research Centre.

Laboratory Investigations:

Estimation of HIV, hepatitis B surface antigen (HBsAg), HBcAb, HBeAg and shistosoma IgG were done by commercially available ELISA kits to exclude the positive cases. ANA, ASMA, AMA and LKM were also measured to exclude immune hepatitis.

The presence of HCV antibodies was determined by third-generation enzyme linked immunosorbent assay (ELISA; CTK-Bioteck-USA), Liver function tests including ALT (alanine transaminase), AST (aspartate transaminase), ALP (alkaline phosphatase) and albumin were assayed using Olympus auto analyser AU400 (Olympus Diagnostica, Japan).

Detection of HCV-RNA by Real Time PCR and HCV Genotyping:

Viral RNA was extracted from patient's plasma using the QIAamp Viral RNA Kit (Qiagen Hilden, Germany, Cat no. 52904) according to the manufacturer's protocol. HCV RNA was detected by commercially available Toyobo RNA-direct real time PCR kit on SLAN Real Time PCR Detection System, LG Lifescience, Korea.

The HCV genotype was defined by the reverse line probe assay (INNO-LIPA v.1.0, innogenetics, Ghent, Belgium) according to the manufacturer's instruction

HLA Class I Typing and KIR Genotyping:

Genomic DNA was extracted from peripheral blood mononuclear cells using the QIAamp DNA minikit (Qiagen) according to the manufacturer's instructions .HLA Class I (A , B ,C) typing was performed by sequence-specific oligonucleotide probe protocols using LABType® SSO Typing kit (ONE LAMBDA,INC): LABType® applies Luminex® technology to the reverse SSO DNA typing method according to manufacturer's instructions.

Genotyping of KIR genes was performed using KIR Genotyping SSP Kit (Invitrogen) according to the manufacturer's instructions.

The primer sets included in the kit amplify the alleles described by the international nomenclature committee of WHO (<http://www.ebi.ac.uk/ipd/kir/>) : 2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DL1, 3DL2, 3DL3, 3DS1, 2DP1, 3DP1.

Statistical Analysis:

Statistical analysis were carried out using "Statistical Package for Social Science (SPSS) Inc., Chicago, IL, USA" (Version 16). Quantitative data was presented in mean and standard deviations (SD), while, qualitative data in number and percent. Analysis of Variance (ANOVA) and Least significant difference (LSD) were used for comparing quantitative means of the three groups. Kruskal Wallis Test and Mann Whitney U were used to compare means of the skeweness data. Chi-square test " χ^2 " was used to compare the qualitative data. Figures were illustrated using excel program.

Results:

This study includes 262 patients with HCV infection; 137 resolved HCV cases and 127 chronic HCV cases. The age of participants ranged between 20-50 years and 228 of the participated cases were males and 34 were females.

The HIV, hepatitis B surface antigen (HBsAg), HBcAb, HBeAg and shistosoma IgG of all the included cases were negative. ANA, ASMA, AMA and LKM of all the included cases were also negative.

Results of HCV genotyping of chronic cases(n=125) is shown in Figure 1, HCV genotype 4 and its subtypes are predominant while genotype 1a is present in 2% of cases.

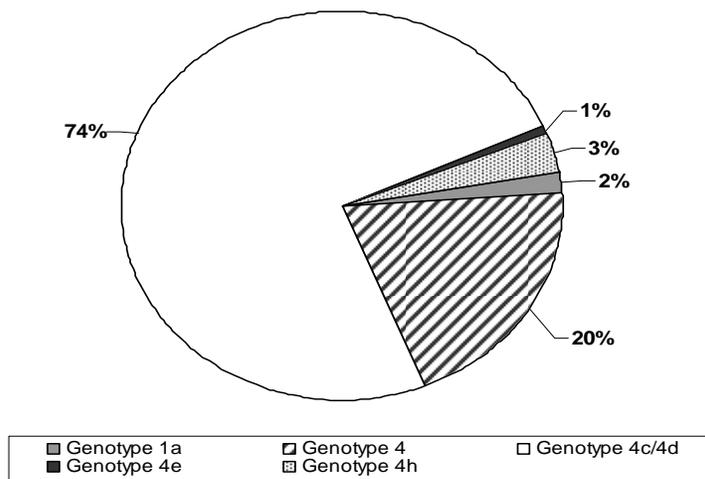


Fig. 1: Results of HCV genotyping using the INNO-LIPA technique.

As regards liver function parameters, ALT AST and albumin of the chronic HCV cases were significantly high compared to that of SVR cases, while there was no significant difference regarding the ALP among different groups (Table 1).

Table 1: Liver function parameters of the study population.

	Resolved HCV (137)		Chronic HCV (125)	ANOVA	
	Spontaneous resolution(17)	SVR (120)		F-ratio	P-value
	Mean ± SD	Mean ± SD			
ALT (U/L)	28±4.77	21.9±1.21	36.8±3.0	10.41	P< 0.0001
AST (U/L)	25.2±1.94	24.6±1.03	33.7±2.65	5.42	P= 0.005
ALP (U/L)	60.8±4.93	69.9±1.90	67.9±2.18	1.24	NS
Alb (g/dl)	4.4±0.38	4.4±0.4	4.1±0.7	8.19	P< 0.0001

SVR: Sustained virological response, Ch: chronic HCV

According to the HLA class I typing results, there was no significant difference in HLA allotypes between chronic and resolved patient groups. Frequency of HLA-B and HLA-C allotypes among studied individuals are summarized in Table (2).

Table 2: Summary of HLA typing in chronic and resolving hepatitis (spontaneous and SVR).

	Chronic HCV	Resolved HCV	χ^2	P-value
	N=125 (%)	N=137 (%)		
HLA-Bw4/Bw4	20 (16%)	23 (16.8%)	0.03	NS
HLA-Bw6/Bw6	46 (36.8%)	36 (24.5%)	3.366	NS
HLA-Bw4/Bw6	59 (47.2%)	78 (56.9%)	2.483	NS
HLA-C1/C1	31 (24.8%)	42 (30.7%)	1.116	NS
HLA-C2/C2	29 (23.2%)	35 (25.5%)	0.195	NS
HLA-C1/C2	65 (52%)	60 (43.7%)	1.764	NS

Table (3) shows KIR-HLA combinations among individuals with chronic and resolved (spontaneous and SVR) hepatitis C. The frequency of individuals homozygous for KIR2DL3 and with two copies of HLA-C1 was higher in the resolved HCV group compared to those with chronic HCV. The frequency of individuals with KIR2DL1 and two copies of HLA-C2 was significantly high in the chronic HCV group compared to the resolved HCV group. Although the frequency of individuals with the activating receptors KIR2DS2, KIR2DS1, KIR3DS1 and their corresponding ligands HLA-C1, HLA-C2 and HLA-Bw4 was higher in the resolved HCV group but this was not statistically significant

Table 3: Summary of KIR-HLA combinations among individuals with chronic and resolved (spontaneous and SVR) hepatitis C.

Genetic factor	Chronic HCV	Resolved HCV	X ²	P-value
	N=125 N (%)	N=137 N (%)		
2DL2/2DL2+HLA-C1/C1	9(7.2%)	3(2.2%)	3.75	NS
2DL3/2DL3+HLA-C1/C1	9(7.2%)	27(19.7%)	8.63	0.003
2DL2/2DL3+HLA-C1/C1	13(10.4%)	13(9.4%)	0.06	NS
2DS2+HLA-C1/C1	6(4.8%)	13(9.4%)	2.14	NS
2DL1+HLA-C2/C2	24(19.2%)	13(9.4%)	5.08	0.02
2DS1+HLA-C2/C2	4(3.2%)	6 (4.4%)	0.25*	NS
3DS1+HLA-Bw4	11(8.8%)	15(11%)	0.34	NS
2DL3/2DL3+HLA-C1/C2	21(16.8%)	17(12.4%)	1.02	NS

*likelihood test is used as more than 25% cells have expected count less than 5.

Discussion:

Natural Killer cells (NK) represent the first line of defense against viral infections. Killer cell immunoglobulin-like receptors (KIRs) on NK cells and their ligands, HLA class I molecules, play an essential part in this tight regulation .

Regarding the HLA-C allotypes , in the present study the frequency of HLA-C1 homozygosity was greater in the resolved group of patients than in the chronic infection group, although the results were not statistically significant. Similar findings were observed in a previous Spanish study (Vidal-Castiñeira *et al.*, 2010), whereas, in a study done by Khakoo *et al.*,2004 on Caucasian and African Americans ,the frequency of HLA-C1 homozygosity was higher in individuals with resolved infections. Khakoo *et al.* (2004) suggested that HLA-C1 homozygosity might have a protective effect on HCV infected hosts, because of the capacity of these molecules to present antigens that have stronger affinities for cytotoxic T cells .

In the current study, the frequency of KIR2DL3/HLA-C1 homozygosity was statistically higher in the resolved group of participants, this finding was also previously reported by Khakoo *et al.* (2004) and Knapp *et al.* (2010). On the other hand , resolution of infection was not associated statistically with the genotypes KIR2DL2/2DL3-HLA-C1 or homozygous KIR2DL2-HLA-C1 coinciding with Khakoo *et al.*(2004) who determined that only individuals homozygous for KIR2DL3, and not KIR2DL2/KIR2DL3 heterozygotes or KIR2DL2 homozygotes, were associated with HCV clearance.

KIR2DL2 binds HLA-C1 with greater affinity than KIR2DL3 (Winter *et al.*, 1998), and has a higher affinity than the stimulatory receptor KIR2DS2 for HLA-Cw3 (HLA-C1 group) due to a single amino acid substitution (Colonna *et al.*, 1997). NK cells will become activated when inhibition is removed, so activation must involve stimulatory receptors (Carrington & Norman, 2003). This explains why individuals with the genotype KIR2DL2-HLA-C1 or KIR2DL2/KIR2DL3-HLA-C1 are more susceptible to persistence of HCV infection.

Furthermore , our study showed a significant increase in the frequency of KIR2DL1-HLA-C2 in the group with persistent infection compared to that of resolved infection. On the other hand ,there was no significant difference between the two groups regarding the frequency of KIR2DS1-HLAC2.

Direct binding of KIR2DS1 to HLA-C group 2 allotypes has been demonstrated (Biassoni *et al.*, 1997), but the reduced level of this binding compared with that of KIR2DL1 has brought into question the physiological relevance of this interaction , it has been suggested that a strong inhibitory interaction could account, in part, for the susceptibility to HCV infection, and the HLA ligand genotype could determine the relative effect of the KIR genotype (Andrew Stewart *et al.*, 2005). Accordingly, Khakoo *et al.*(2004) reported a higher frequency of HLA-C2C2 genotype among patients with persistent infection and a higher frequency of HLA-C1C1 in individuals with resolved infection.

Conclusion:

Our results support a role for the HLA/KIR in determining the outcome of HCV infection. In the current study, viral resolution was associated with KIR2DL3/HLA-C1 homozygosity, while virus persistence was associated with KIR2DL1/HLA-C2/C2.

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