ORIGINAL ARTICLE

Hepatitis Monthly 2008; 8(1): 11-16

Epidemiology of Chronic Hepatitis C Virus Infection in High Risk Groups

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Background and Aims: **The relative frequency of hepatitis C virus (HCV) genotypes and differences of HCV genotype distribution in relation to transmission mode, age and acquisition time of infection were investigated among 758 high-risk patients: 118 were under dialysis (DP), 109 intravenous drug users (IDU), 317 hepatologic (HP) and 214 β-thalassemic patients (βTM).**

Methods: **A total of 478 anti-HCV (Enzyme Immunoassay EIA) positive sera were further examined for HCV-RNA (Transcription Mediated Assay-TMA), HCV genotype (Line Probe Assay-LiPA) and hepatitis C viral load (branched DNA-bDNA). With the exception of IDU group, patients were contaminated before 1990 after blood transfusion or other nosocomial treatment. For IDU, who were significantly younger individuals than DP and HP, HCV infection occurred the last decade after IV drug use.**

Results: **The overall distribution of HCV genotypes 1, 2, 3 and 4 was 38.6%, 8.4%, 40.7%, and 12.4%, respectively. With the exception of IDU group in which genotype 3 was by far the most common, genotype 1 predominated in all patients examined.**

Conclusions: **Results reveal a difference of HCV genotype distribution among patients of high risk groups in our area, as a sequel of epidemiologic changes in HCV transmission. IDU group may be also responsible for a new hepatitis C epidemic and may constitute the new significant reservoir in the future. This transition may require new prevention protocols and therapeutic strategies.**

Keywords: **Post-Transfusion Hepatitis C, HCV Genotypes, HCV Epidemiology**

Introduction

Hepatitis C virus (HCV) is a positive-stranded RNA agent identified as the main cause of post-transfusion non A, non B hepatitis (PTH) before routine blood screening early in 1990. The genome of HCV is highly variable. So far, six major genotypes and more than one hun-dred subtypes have been described [(1)]. This long genetic evolution of genotypes may involve variable pathogenic patterns.

Many studies have indicated an association between HCV genotype and responsiveness to alpha interferon treatment [(2, 3)]. Differences in geographic distribution of HCV genotypes have also been observed [(4-7)]. Association of HCV genotype prevalence to the risk factors and the mode of HCV transmission has been also reported [(8-10)]. After 1990, systemic screening of blood for anti-HCV has led to a dramatic decrease of hepatitis C as PTH [(11)]. In underdeveloped or developing countries acquisition of HCV infection is mainly nosocomial as a sequel of low health care standards [(12, 13)]. The major route of HCV infection in devel-oped countries is through the use of intravenous drugs [(14)]. In these countries combined ther-apy with pegylated interferon and ribavirin has favorable results for HCV genotype 2 and 3 [(15-17)] but influences minimally the genotype distribution.

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Received: *6 Feb 2008* ***Revised:*** *29 Feb 2008*
Accepted: *8 Apr 2008*

Hep Mon 2008; 8 (1): 11-16

In order to study chronic hepatitis C and better understand differences in the epidemiology of HCV infection and HCV genotype patterns among high risk groups of Southwestern Greece, the relationship between HCV genotypes, viral load, risk factors, modes of transmis-sion and acquisition time of infection among such chronic hepatitis C patients of different ages was evaluated.

Materials and Methods

Patients

Serum samples of 758 high-risk for HCV infection consecutive patients (467 men and 291 women, 18 to 60 years of age) from different departments of our hospital, during 2001 to 2004 were included in this study. Patients'sera were examined for hepatitis C at the Labora-tory of Microbiology and were assigned to four groups: A) 118 dialysis patients (DP), 74 men and 44 women, 28 to 60 years of age, with mean duration on dialysis treatment of 105±58 months. These patients acquired chronic hepatitis C, contaminated by intravenous catheters or other equipment and solutions shared by several patients before 1990. In addition a history of past blood transfusion (before 1990) was elicited from 84 (80%) DP patients. All of them were vaccinated for hepatitis B; B) 317 hepatologic patients (HP), 178 men and 139 women, 28 to 60 years of age. These patients were in regular follow up at the hepatology clinic due to transaminasemia. The most likely mode of HCV transmission was also parenteral exposure. About 53% of HP had a history of pre-existing hospital admission before 1990, including blood transfusion, whereas 150 (47%) had probable a different nosocomial source of infection with parenteral exposure to blood (intravenous infusion, surgery, contaminated equipment) before 1990 also. Exclusion criteria for HP group were: dialysis patients, intravenous drug users (IDUs) and multitransfused adults with β-thalassemia (βTM); C) 109 prior IDUs, all male prisoners, 25 to 35 years old. They were studied prospectively after cessation of intrave-nous drug use. Drug use occurred the last decade and had duration of 4 to 6 years for each person, according to their clinical information. The most probable year of HCV infection was conventionally accepted as the first year of drug use. Sharing of contaminated needles was the main risk factor in this group; D) 214 multitransfused adults with βTM, 106 men and 108 women, 18 to 30 years old, transfused since their childhood, as part of their therapy. All of them were vaccinated for hepatitis B. Patients with PTH in DP, HP and βTM groups received blood or blood products prior to implementation of routine screening for HCV (1990). All studied subjects were of Greek origin.

Methods

Serum samples were aliquoted and stored at -70°C until use. A third generation micropar-ticles enzyme immunoassay MEIA-Axsym (HCV (V3) MEIA, Abbott, Germany) was used to screen patients' sera for anti-HCV. Among 758 tested sera, 478 (63.1%) were anti-HCV posi-tive, showed elevated serum alanine aminotransferase (ALT) levels for more than six months and fulfilled the criteria of chronic hepatitis C (CHC). Hepatitis B surface antigen (HBsAg) was positive in 8 (1.6%) patients with CHC while there was no evidence of HIV coinfection. Both serological markers HBsAg and anti-HIV were determining by microparticles enzyme immunoassay MEIA-Axsym (HBsAg (V2) MEIA, Abbott, Germany, HIV1/2 gO MEIA, Ab-bott, Germany). Anti-HCV positivity in 66 patients with negative HCV-RNA was confirmed by a supplementary blotting line immunoassay INNO-LIA (INNO-LIA HCV Innogenetics, Belgium). None of them had a history of alcohol abuse, none of them was on hepatotoxic me-dication and the tests for autoimmune hepatitis were negative.

HCV-RNA was detected in 412 out of 478 sera by Transcription-Mediated Amplification (TMA), an isothermal nucleic acid amplification technique (Versant HCV-RNA qualitative assay Bayer diagnostics, Emeryville, CA) which relies on reverse transcriptase and T7 RNA polymerase to generate RNA and DNA amplicons [18]. The lower sensitivity cut-off of TMA is 5.3 IU/mL. HCV genotyping was carried out by a line probe assay, LiPA (HCV genotype assay LiPA, Versant, Bayer, Tarrytown NY, USA), a reverse hybridization test that can asso-ciate the 5' NCR genome in products of a post PCR amplification of DNA-TMA amplicons. This assay allows differentiation of 6 major HCV genotypes (1-6) and 15 subtypes (1a, 1b, 2a/2c, 2b, 3a, 3b, 3c, 4a, 4b, 4c4d, 4e, 4f, 4h, 5a, 6a) according to Simmonds *et al* [19]. Sub-type 1a/1b was referred as genotype 1, while 2a/2c as genotype 2 in this study. All genotypes 2 were subtyped as 2a/2c, whereas all genotypes 3 were subtyped as 3a. All viremic cases were genotype classifiable. HCV-RNA was quantitated by a branched oligodeoxyribonucleo-tide (bDNA) signal amplification assay (HCV-RNA 3.0 Assay, bDNA, Versant, Bayer, Tarry-town NY, USA) and expressed as international units per milliliter of serum

(IU/mL). Geno-typing and quantitation of HCV-RNA was performed in all viremic patients before starting of any treatment. Some of the patients later received antiviral treatment, while others did no.

Statistical Analysis

Statistical analyses were performed using SPSS 12.0 (SPSS Inc.) for Windows. Prevalent HCV infections included all cases that were found anti-HCV positive. To describe the general characteristics of the four risk groups, frequencies were calculated for categorical variables and means were calculated for continuous variables. To compare anti-HCV positive cases as well as the distribution of the different genotypes among the single risk categories, chi-square tests, prevalence odds ratios, and 95% confidence intervals (CI) were performed. Two-sided P values were used and P values less than 0.05 were considered to be statistically significant.

Results

A total of 758 sera of patients divided in four groups were positive for anti-HCV antibod-ies. The average age and percentage of male vs. female were different among studied groups. The main demographic, biochemical, virologic and genotypic characteristics of all high-risk groups are shown in Table 1. The seroprevalence rate (positive anti-HCV) was 75.2% in the IDU group, 65.3% in patients on hemodialysis, 64% in patients with hepatologic diseases and 54.2% in patients with βTM. The prevalence of anti-HCV was significantly higher in IDU as compared to HP (75.2% vs. 64%, P=0.032) and βTM group (75.2% vs. 54.2%, P<0.001). Mean age and age distribution did not differ between DP and HP (55.9 vs. 54.9) or between IDU and βTM (27.3 vs. 21.9) while individuals of IDU and βTM were significantly younger (P<0.001) than those of DP and HP groups, with different age distributions.

Taking into account all HCV-RNA positive cases (412), genotypes 1 (38.6%) and 3 (40.7%) were most common, while only 12.4% and 8.4% carried genotype 4 and 2, respec-tively. However, within each risk group a different genotype distribution was observed (Figure 1). Genotype 1 predominated among DP and HP groups. A statistically significant differ-ence was observed in the prevalence of this genotype between the DP (47.0%) and HP (45.1%) groups as compared to IDU (30.1%, P<0.05) and βTM (32.0%, P<0.05) individuals, nevertheless the incidence of subtype 1a versus 1b was significantly higher in IDU (38.8%) as compared to the DP (19.3%, P<0.01) and HP (19.4%, P<0.01) groups. Genotype 3 was more common in the IDU group in relation to DP group (53.4% vs. 40.9%) and to HP group (53.4% vs.40.5%), while was significantly higher in relation to βTM group (53.4% vs. 28%, P=0.003). In contrast to the overall distribution pattern, genotype 4 was by far the second most common one among patients with βTM as compared to DP, IDU and HP groups (30% vs. 6.1%, 8.2 and 5.2, P<0.001, respectively).

βTM patients were multitransfused early in their

Table 1. Demographic, biochemical, virologic and genotypic characteristics of the study population (n=758).

	Dialysis patients (DP)	Intravenous drug users (IDU)	Hepatologic patients (HP)	β-thalassemia major patients (βTM)
No.	118	109	317	214
Male gender (%)	74 (62.7)	109 (100)	178 (56.2)	106 (49.5)
Age, mean (years)	55.9±7.0	27.3±3.0	54.9±8.3	21.9±2.5
Age, range (years)	28-60	25-35	28-60	18-30
ALT, mean (IU/L)	50.1±20.3	69.2±29.2	73.1±10.4	64.2±24.4
HCV status positive (%)	77(65.3)	82 (75.2) [a, b]	203 (64.0) [a, c]	116 (54.2) [b, c]
anti-HCV (+) & HCV-RNA (+)	66 (55.9)	73 (67.0)	173 (54.6)	100 (46.7)
anti-HCV (+) & HCV RNA (-)	11 (9.3)	9 (8.3)	30 (9.5)	16 (7.5)
HCV status negative (%)	41 (34.7)	27 (24.8)	114 (36.0)	98 (45.8)
Male HCV (+) (%)	50 (65.0)	82 (100.0)	114 (56.2)	57 (49.1)
HCV-RNA mean level (IU/mL)	340×10^3	728×10^3	779×10^3	360×10^3
Genotypes (%)				
▶ 1 (1a,1b,1a/1b)	31(47.0) [d]	23 (30.1) [d,e]	78 (45.1) [e,f]	32 (32.0) [f]
▶ 2 (2a/2c)	4 (6.1)	6 (8.2)	16 (9.2)	10 (10.0)
▶ 3 (3a)	27 (40.9)	39 (53.4) [g]	70 (40.5)	28 (28.0) [g]
▶ 4 (4,4h)	4 (6.1) [h]	6 (8.2) [i]	9 (5.2) [k]	30 (30.0) [h, i, k]

[a] 75.2% vs 64%, P=0.032; [b] 75.2% vs 54%, P<0.001; [c] 64% vs 54%, P=0.023; [d] 47% vs 30.1%, P=0.041; [e] 30.1% vs 45.1%, P=0.048; [f] 45.1% vs 32%, P=0.033; [g] 53.4% vs 28%, P=0.003; [h] 6.1% vs 30%, P<0.001; [i] 8.2% vs 30%, P=0.001; [k] 5.2% vs 30%, P<0.001

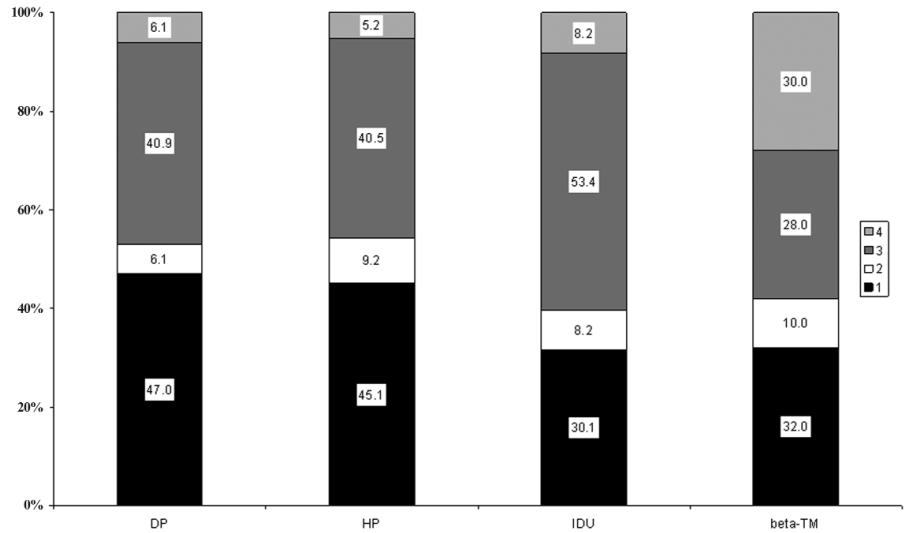

Figure 1. HCV genotypes distribution according to the different risk groups.

childhood while DP and HP had shown hospital admissions for dialysis and/or blood transfusion before the implementation of routine screening of HCV (1990). IDU were young people contaminated with HCV the last ten years by needle sharing. Mixed HCV infections with two or more HCV genotypes were not identi-fied in any case. DP and βTM patient had significantly lower serum HCV-RNA load (340,000-360,000 IU/mL) as compared to IDU and HP groups (728,000-779,000 IU/mL).

Discussion

Realization that chronic HCV infection leads to clinical sequel of liver cirrhosis and hepa-tocellular carcinoma renders control of hepatitis C as one of the most significant public health challenges in the last decade. Before 1990, blood transfusion was associated with a high risk of HCV infection [20]. After systemic screening of blood, post-transfusion hepatitis C has de-creased dramatically [11]. High health standards in hospitals of developed countries has also led to a dramatic decrease of nosocomial hepatitis C, whereas combined treatment with inter-feron and ribavirin has substantially increased the sustained virologic response in patients with chronic HCV infection [15, 16]. The main transmission route of hepatitis C in such countries is through the use of intravenous drugs [14].

The high rate of anti-HCV (>54%) in all high-risk groups in the present study is in accor-dance with the literature [10, 21-23]. IDU patients had significantly higher incidence of hepa-titis C as compared to HP (P=0.032) and to βTM (P=0.001) groups. This may be due to the fact that this group, composed of significant younger patients, usually take no health control measurements, and were contaminated the last decade by needle sharing. The high rate of an-ti-HCV positivity in DP (65%), HP (64%) and βTM (54%) patients suggests that major expo-sure to HCV occurred many years ago, before1990, when HCV infection was more wide-spread, as it was unknown and uncontrolled. The fact that these patients received blood prod-ucts prior to the implementation of routine screening for HCV explains our findings [21, 24].

Within hemodialysis and nosocomial patients besides handling of intravenous catheters, another potential source of HCV infection is exposure to HCV contaminated equipment, and/or to HCV contaminated multidose vials [25, 26]. Thus, horizontal nosocomial patient to patient transmission of HCV probably occurred in the past as another means in DP, HP and βTM groups. The risk of infection in hemodialysis patients appears to correlate with duration of hemodialysis and the number of transfusions [27-29]. As HP, DP, βTM included patients contaminated before 1990 by blood transfusion or other parenteral exposure to blood, it is of interest that IDU patients with a different source of HCV infection that were contaminated during the last decade, included individuals significantly younger than DP and HP. IDU re-mains the group with the higher rate of anti-HCV representing the main new continuity of HCV reservoir in our area, as it has been recently observed in many other developed countries [30].

Geographic variability of HCV genotypes exists and relates to the route and age of infec-tion. Genotypes 1, 2 and 3 are widely distributed [5, 14, 16], while genotype 4 has been found to northern and central Africa [6] and in Mediterranean countries. Genotype 1 is the most fre-quent

genotype worldwide [5, 13]. Favorable treatment outcomes (>80%, in six months), ob-served mainly in patients infected with genotype 2 and 3 [15-17], affect minimally the geno-type distribution. Genotype 3a has been commonly identified among young population in Western countries, especially in drug users [31, 32].

Data from the present study reveals that genotype 1 (38.6%) and 3 (40.7%) are the pre-dominant genotypes among chronically infected high-risk patients. Genotype 4 is detected in 12.4% of these patients, followed by genotype 2 (8.4%). However, distribution of HCV geno-types was different between IDU and other groups tested (DP, HP, βTM). Genotype 1 pre-dominates among patients that acquired HCV infection by blood transfusion and other par-enteral exposures before 1990. Subtype 1a vs.1b was significantly higher in IDU in relation to DP and HP groups. This is in accordance with literature [31]. In the elderly patients of DP and HP groups the second most common genotype was 3 (41%), whereas in younger transfused patients of βTM group, genotype 1 (30%) was followed by 4 (30%) and 3 (28%). Genotype 4 (30%) approaches the highest percentage reported among multitransfused populations in Eu-rope and USA [33, 34]. The most probable explanation for this interesting observation is that genotype 4 was imported from African countries by returning Greek expatriate communities who lived there for many decades. An unnoticed nosocomial HCV genotype 4 outbreak be-fore 1990 in βTM unit may be another possible explanation for the high prevalence of geno-type 4 observed in this group.

In accordance with many studies of other European countries [29, 35, 36], the predomi-nant HCV genotype among IDU is type 3 (54%). This group consists of chronic infected males of Greek origin, significantly younger than DP and HP, who acquired HCV infection by drug injection the last decade. Genotype 3 was more common in IDU, in relation to DP and HP group (53.4% vs. 40.9% and 40.5%, respectively), while it was significantly higher as compared to βTM patients (53.4% vs. 28%, P=0.003). This finding suggests that genotype 3 plays a crucial role in this separated, young people's group who had sharing of contaminated needles as the main and special contamination route. IDU group may be also responsible for a new hepatitis C epidemic that is not as yet under obliged control and may constitute the new significant reservoir in the future. It is of surprise to observe that nobody of IDU had mixed genotype infection. Indeed, since intravenous drug use is the major risk factor of new hepatitis C cases at the present time, re-exposure is expected to increase the number of mixed infec-tions. A possible explanation of this phenomenon is that IDU patients with prior HCV infec-tion appeared to be ¨protected¨ or they were more resistant against further infection with HCV despite repeated exposure [36, 37]. However in another study it was shown that T-cell medi-ated protective immunity to HCV is strain specific and despite their ability to clear one HCV strain, patients may be re-infected with a heterologous strain that can then persist [38].

DP (n=118; 340 x10^3 IU/mL) and βTM (n=214; 360 x10^3 IU/mL) patients had a signifi-cantly lower viral loads as compared to HP (n=317; 779 x10^3 IU/mL, P<0.05) and IDU (n=214; 728 x10^3 IU/mL, P<0.05). The lower HCV-RNA levels in DP, is mainly attributed to the destruction of HCV particles by dialyzer membranes during hemodialysis [39]. A possible explanation for the same finding in βTM group could be the increased number and activity of suppressor T-cells (CD8) in chronically transfused βTM patients [40, 41]. It has been shown that a stronger polyclonal CD8 cytotoxic T-cell response in the peripheral blood and the liver is associated with lower levels of HCV viremia [42]. Observed differences of genotype distri-bution among the specific high-risk patients, main in IDU, are of importance since the high frequency of genotype 3 may lead to minimization of HCV reservoir as patients respond fa-vorably to a six month therapy protocols. Moreover, application of more effective health con-trol programs [21, 23] for IDU in our area, may lead to control of new intravenous drug cases of hepatitis C.

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