ORIGINAL ARTICLE

Significant epidemiological changes in chronic hepatitis C infection: results of the nationwide HEPNET-GREECE cohort study

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Abstract

Background and Aims: Hepatitis C virus (HCV) infection is an important health problem worldwide. The aim of the study is to describe the baseline characteristics and possible epidemiological changes of the patients with chronic HCV infection included in a nationwide Greek study.

Patients and Methods: two thousand eight hundred seventeen (2817) patients, followed-up at 20 hepatology centres throughout Greece between the years 1997 and 2006 were enrolled in the study.

Results: Intravenous drug use (IDU) and history of blood transfusion prior to 1992 was reported in 30.7% and 22.6% of our patients, respectively. In 1865 (66.2%) patients with known genotypes, the distribution for genotype 1, 2, 3 and 4 was 45.1%, 7%, 34% and 13.9% respectively. Genotype 1 was more common in older people, in women (55.9% $p<0.001$) and patients with transfusion-related hepatitis (61.6% $p<0.001$). Genotype 3 was more common in younger patients, in men (43% $p<0.001$) and in IDUs (63.3% $p<0.001$). A significant reduction of transfusion-related hepatitis C incidence ($p<0.001$) in conjunction with the proportion of genotype 1 ($p<0.001$) was observed during the last three decades while an increase in IDU infected patients and genotype 3 was detected.

Conclusions: Our study showed a significant change in HCV genotype distribution and source of HCV infection during the last three decades and under that scope, urgent actions are needed in order to control the spread of HCV infection. Hippokratia 2011; 15 (1): 26-31

Key Words: hepatitis C epidemiology, Greece, HCV genotypes, transfusion, intravenous drug use

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Hepatitis C virus (HCV) infection is an important public health problem worldwide. HCV-infected people serve as a reservoir for transmission and are at risk for developing cirrhosis and hepatocellular cancer (HCC). Thus, in order to address the problem of HCV infection, there is a need to assess the burden of disease in each country and subsequently implement preventive and therapeutic strategies[1,2].

Although the exact prevalence of HCV infection in Greece is not well known, it is estimated that approximately 2% of the general population have chronic HCV infection with a wide geographical variance of seropositivity (0.5%-7.5%)[3-6]. In a recent evaluation performed in Greece, it was estimated that HCV-related morbidity and mortality will increase during the following 20-30 years[7]. However, special issues such as the true incidence of HCV infection in the Greek population, the contamination risk factors distribution, the natural course and long-term outcome of the disease in addition to the possible changes during time, remain unknown.

HEPNET - GREECE (Hepatitis Network - Greece) for hepatitis C, is a nationwide retrospective-prospective study, initiated in 2003**.** The study was sponsored by the Greek government, approved and conducted through the Hellenic Center for Diseases Control and Prevention (HCDCP, KEELPNO, Greece). The main aims of the study are to evaluate the epidemiology and course of chronic hepatitis C infection in Greece and their longitudinal changes. In this initial report, patients with HCV infection, their baseline demographic, clinical and virological characteristics at their initial presentation at a hepatology centre are described. The possible epidemiological chang-

es, focusing on HCV genotypes and route of transmission that may affect not only the daily routine clinical practice but also the public health strategies are also described.

Patients and methods

The HEPNET-GREECE network was established in 2003 aiming to collect and evaluate data regarding patients with chronic viral hepatitis B and C in Greece. Twenty hepatology centres throughout the country participated in the HEPNET-GREECE study. All anti-HCV positive individuals followed in the above centers that met the following inclusion criteria were enrolled in the study: 1. Anti-HCV positivity and detectable serum HCVRNA for at least 6 months 2. All patients who were under follow up on 01/01/1997 (i.e. at least two visits available), regardless of treatment and final clinical outcome, or patients who initiated their follow-up at the participating centers between 01/01/1997 until the end of June 2006. Subjects co-infected with HIV or HBV were excluded from the study. For the present analysis, patients aged below 14 years at registration were also excluded.

A structured case electronic record form (CRF) was used for data collection. Prior to this network establishment (i.e. for the period 1997-2003) data were collected retrospectively from patients' medical records and were prospectively updated twice a year thereafter.

Study entry was considered as the date of patient's first visit to the center. The follow-up time was defined as the time interval between study entry and last available patients' clinical information. At study entry, detailed data on patients self-reported demographic characteristics were collected as well as clinical signs, other chronic diseases and treatment history. Biochemical, virological, histological, serological and ultrasounds' findings were also recorded.

For HCV-RNA determination at study entry, PCR was used in 90.22% (n=2020) of the patients qualitative (HCV Combas-Amplicor, Roche Diagnostics) in 56.4%, quantitative (Amplicor HCV Monitor, Roche Diagnostics) in 43.6%), bDNA (Versant HCV, Siemens) in 3.13% (n=70), other assays in 2.46% (n=55) and in 4.20% (94) the method used was unknown. For genotype determination the Versant Genotyping HCV (Siemens) was used.

Diagnosis of cirrhosis was based on histological findings, provided that liver biopsies were conducted within 6 months from study entry. Duration of the infection was defined as the time interval between the date of study entry and date of infection (i.e. the first intravenous drug use or blood transfusion provided that it was prior to 1992). Ethnicity was defined based on country of birth. For the analysis, Greeks and those born in Western Europe were classified as non-immigrants, whereas the remaining of the study population was classified as immigrants.

Statistical analysis

Descriptive statistical analysis was performed for baseline patients' characteristics at first visit. Results were expressed as absolute and relative frequencies for qualitative variables and as medians and interquartile ranges for continuous variables. Comparison of categorical variables was done by the X^2 test. For the comparison of continuous variables in subgroups of the study population the non-parametric Mann-Whitney test was used. P-values less than 0.05 were considered as statistical significant. Multivariable multinomial logistic regression was applied to test for time trends and to identify significant independent prognostic factors of, the relative proportion of each HCV genotype.

Results

In total 2996 patients with HCV infection were enrolled in the study. One hundred and fourty-three (143) patients were excluded as being HCV-HBV co-infected, 30 were <14 years at registration and 6 had unknown date of birth. Therefore, 2817 patients consisted the study population for the present analysis.

Demographic and Clinical Characteristics

The baseline characteristics of the study population are shown in Table 1. The majority of our patients were male, born in urban areas and well educated, with a median age of 41.3 years, about half of them were over-weight or obese, 44% were current smokers and 14.8% immigrants [the majority of them were from Eastern Europe (n=197; 54.6%), followed by Egyptians (n=59; 16.3%) and Albanians (n=39; 10.8%)]. History of acute icteric hepatitis was reported in 6.0% and one third (29.5%) presented with at least one other chronic disease such as hypertension (11.6%), diabetes mellitus (6.4%), cardiovascular disease (6.0%) and neoplasia (0.9%). In total 7.2% of the patients had a treatment history with antivirals (65.2% with IFN monotherapy and 34.8% with IFN and Ribavirin combination) before their baseline visit to the center.

Clinical signs of advanced liver disease at presentation such as splenomegaly, ascites, flapping tremor and icterus were observed in 11.7%, 2.6%, 0.9% and 1.6%, respectively. The stage of disease at study entry based on histological evaluation, was available for 1361 (48.3%) patients. A total of 196(7.0%) patients were classified as cirrhotics. In 80/196 patients with cirrhosis, the date of study entry and HCV diagnosis was identical. Cirrhotics were older than non-cirrhotics (median (IQR) age: 56.5 (44.2-64.8) vs 37.6 (29.5-51.3) years, $p<0.001$), whereas 40/196 had a history of treatment prior to study entry. Furthermore, one hundred eighteen (4.2%) patients presented with advanced disease at study entry. In particular, 97 had decompensated cirrhosis and 21 HCC.

Route of Transmission and Genotype Distribution

Intravenous drug use was reported in 30.7% of our patients and 22.6% had a history of blood transfusion prior to 1992. Other possible sources (dental procedures, surgery, occupational exposure etc) were reported in smaller rate (10.1%), while for 36.6% of the patients, the route of transmission was unknown. Compared to those

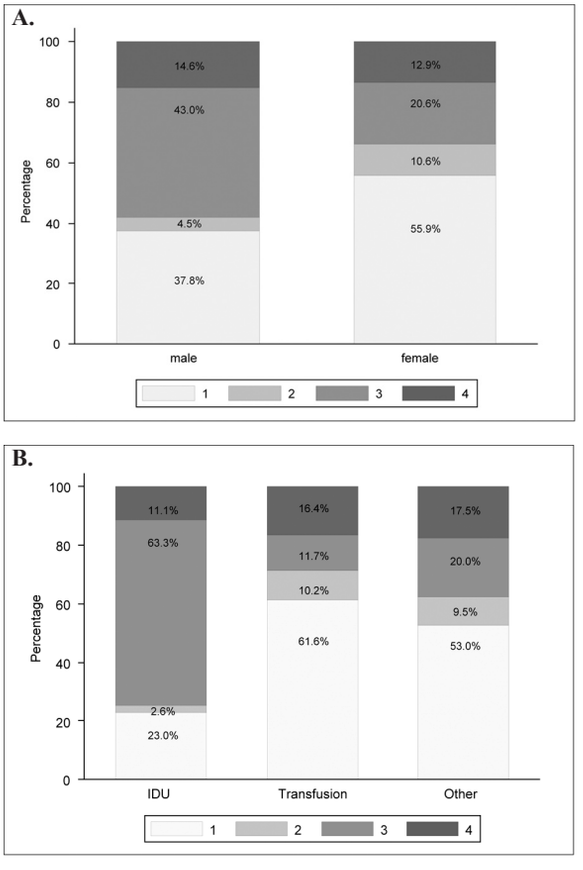
Table 1: Baseline characteristics of the study population.

Gender, Male, (n,%)	1670	59.3
Birth Place, (n,%)		
Rural	519	23.8
Semi-urban	312	14.3
Urban	1351	61.9
Residence Place, (n,%)		
Rural	277	10.3
Semi-urban	205	7.6
Urban	2215	82.1
Ethnicity		
Non-immigrants	2,086	85.2
Immigrants	361	14.8
History of acute icteric hepatitis, (n,%)	166	6.0
Chronic diseases, (n,%)	813	29.5
BMI (kg/m2), (n,%)		
<25	1074	50.8
25-30	794	37.5
>30	247	11.7
Education, (n,%)		
Illiterate/elementary school	583	28.6
Secondary school	1010	49.5
Higher education	449	22.0
Smoking, (n,%)		
No	1111	45.5
Ex-smoker	246	10.1
Current smoker	1083	44.4
Cirrhosis at study entry, (n,%)	196	7.0
Age at study entry, median (IQR)	41.3	(30.7, 56.1)
ALT, Normal (n,%) (Lower than the upper normal limit or lower than 40 IU/L if missing)	569	25.5
Total bilirubin, ≥1.1 mg/dL (n,%)	373	20.4
Genotype, (n,%)		
1	841	45.1
2	130	7.0
3	634	34.0
4	260	13.9

infected via other route, IDU's were more frequently male, from urban areas as opposed to rural or semi-rural areas, with higher education, younger at study entry and less frequently immigrants. Demographic characteristics of the patients with unknown route of transmission were more similar to those infected through another route than IDU.

HCV genotype was available in 1865 subjects (66.2%). Hepatitis C virus genotype 1 was the most common in our study (n=841, 45.1%); genotype 2, 3 and 4 were found in 130 (7.0%), 634 (34.0%) and 260 (13.9%) patients, respectively. For 63 subjects, HCV genotype could not be indentified. Hepatitis C virus genotype distribution differed significantly (p<0.001) by gender with genotype 1 being more frequent in women than in men (55.9% vs 37.8%), and genotype 3 more frequent in men (43.0% vs 20.6%) (p<0.001). The frequency of genotype 4 was similar in both genders (men/women: 14.7% / 12.9%). (Figure1A). Genotype distribution differed between Greeks and immigrants (Greek/immigrants: 45.2% vs 49.8%; 6.9% vs 8.8%; 34.6% vs 22.6% and 13.2% vs 18.8% for genotype 1, 2, 3 and 4 respectively; p=0.001) Among the 49 immigrants with HCV genotype 4, 38 (77.6%) were born in Egypt.

Furthermore, a significant difference (p<0.001) of HCV genotype distribution by the source of infection was



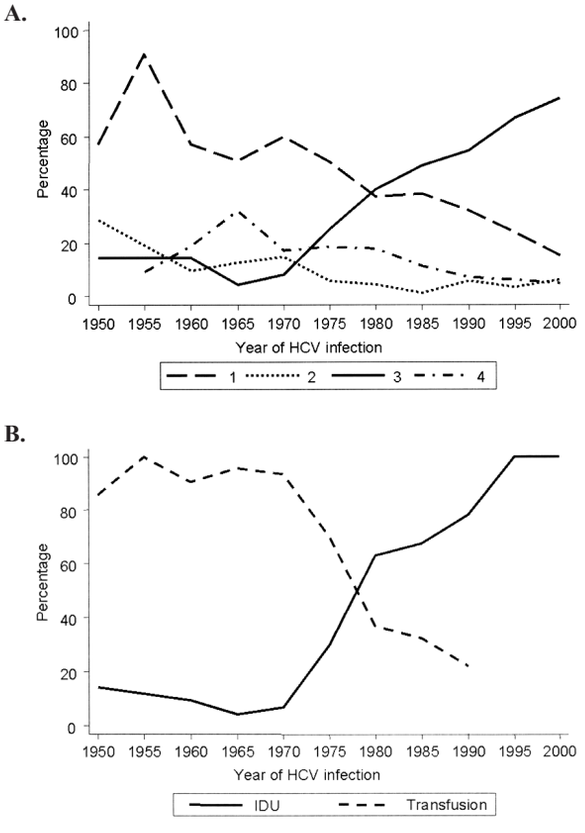
1. Figure 1: Distribution of hepatitis C virus genotype by gender **(A)** and source of infection **(B)**.

observed. Genotype 1 was found more frequently among patients with a history of blood transfusion than among those with IDU's (61.6% vs 23.0%). On the contrary, patients with a history of drug use had more often genotype type 3 HCV infection compared with those who had history of blood transfusion (63.3% vs 11.8%). In patients with other sources of infection genotypes type 1 and 3 were found in 53.0% and 20.0%, respectively (Figure 1B).

Time Changes of Genotypes Distribution and Routes of Transmission

In 986/1865 patients with known HCV genotype the probable date of infection could be estimated. A significant association was identified between HCV genotype and time of disease acquisition. A reduction in the proportion of subjects with genotypes 1 or 4 and a sharp increase in the proportion of subjects with genotype 3 was observed over time ($p<0.001$; Figure 2A). In patients infected with genotype 1, a longer duration of infection was observed compared with those infected with others genotypes (median 21.1 vs 15.2 years, respectively; $p<0.001$).

Similarly, we also observed a significant association between the source of infection and duration of the disease. A significant reduction in transfusion-related hepatitis C after 1980 was observed over time. On the contrary there was a dramatic increase in IDU related hepatitis C, mainly after 70's ($p<0.001$; Figure 2B).



2. Figure 2: Distribution of (A) HCV genotypes and (B) source of infection by time.

In addition, a significant difference of genotype distribution based on age at infection ($p<0.001$) was observed. Genotype 1 was less common in patients <25 years of age, than in those ≥25 years old (33.7% vs 48.2%), whereas, genotype 3 was less common in patients ≥25 years old (31.0% vs 49.0%). Similarly, the source of infection differed significantly according to age at infection ($p<0.001$), with a median (IQR) age of 19.8 (17.2-23.5) years for IDU and 26.0 (17.0-36.5) years for blood transfusion.

Results from multivariable multinomial logistic regression showed that compared to having HCV genotype 1, the probability of having HCV genotype 2 remained relatively stable over time and did not differ significantly by either route of infection or gender; the probability of having HCV genotype 3 increased by about 23% per 5 years, was almost 7 times higher in IDUs compared to those infected through blood transfusion and higher in males than in females by about 53%; the probability of having HCV genotype 4 decreased by about 11% per 5 years, was more than twice higher in IDUs than in those infected through blood transfusion, whereas did not differ by gender. Age at HCV infection did not affect significantly the probability of HCV genotype after adjusting for year or route of infection and gender (Table 2).

Discussion

The main finding of this multicenter national Greek study is the substantial changes in genotype distribution of HCV infection over the last 30 years in Greece. According to our data, the significant increase of genotype 3 and the decrease of genotype 1 infection were mainly correlated to changes in the mode of HCV transmission, gender and time of HCV infection acquisition.

Age at HCV infection, although found to be associated with the changes in HCV genotypes' distribution over time in univariable analysis, its significance did not persist in multivariable analysis. This is because, the younger HCV positive patients belonged to the group of IDU acquired infection, while the older ones belonged to the blood transfusion acquired infection. It is worth mentioning that the risk of transfusion-related HCV-hepatitis progressively declined during 1980s and 1990s possibly due to the implementation of an all-volunteer blood donor system and the effective virus-inactivation procedures for blood derivatives. It should be noted that in Greece the blood system started screening donations with the first generation EIA in 1992 whereas the 2nd and 3rd generation EIA were introduced in 1994 and 1999 respectively

Furthermore, through the evaluation of patients with known genotype and a defined duration of HCV infection, we recorded a significant increase in the proportion of patients infected with genotype 3 mainly after the 80s and a decline of post-transfusion HCV infection over time. Therefore, blood safety and improvement in infection control practices have paved the way for IDU to become not only the main risk factor for HCV transmission but also to alter the genotype distribution among patients with HCV hepatitis.

Table 2: Probability of having HCV genotype 2, 3, or 4 compared to having HCV genotype 1. Results from multivariable multinomial.

	RR	95% CI	P
Genotype 2			
Year since HCV infection	1.076	0.907 - 1.276	0.402
(per 5 years)			
Route of infection	0.582	0.258 - 1.309	0.190
IDU/transfusion			
Gender	0.589	0.319 - 1.088	0.091
Male/female			
Age at HCV infection	0.820	0.644 - 1.044	0.108
(per 10 years)			
Genotype 3			
Year since HCV infection	1.234	1.113 - 1.369	<0.001
(per 5 years)			
Route of infection	6.937	4.339 - 11.091	<0.001
IDU/transfusion			
Gender	1.527	1.057 - 2.208	0.024
Male/female			
Age at HCV infection	0.878	0.735 - 1.048	0.150
(per 10 years)			
Genotype 4			
Year since HCV infection	0.887	0.789 - 0.997	0.044
(per 5 years)			
Route of infection	2.166	1.249 - 3.754	0.006
IDU/transfusion			
Gender	1.154	0.741 - 1.799	0.526
Male/female			
Age at HCV infection	0.980	0.809 - 1.188	0.839
(per 10 years)			

Recent studies from France and Italy have also shown a decrease in genotype 2 and 1b and the emergence of genotype 3[8,9]. In a previous study, although conducted in a single center including a population sample not representative of the whole country, the evaluation of the relative frequencies of HCV genotypes in 434 unselected Greek patients, showed a similar trend in genotype distribution[10].

According to the 2006 National report of the Greek Reitox Focal Point (EKTEPN) and the European Monitoring Center of Drugs and Drug Addiction (EMCDA) (http://www.emcdda.europa.eu/publications/national-reports), the number of IDUs showed a 14.1% increase in 2006 compared to 2005 with a 12.1% increase in the total of IDUs whose primary drug dependence is with heroin. Although a decrease of injecting use and syringe sharing rates was reported for 2006, the overall rate of sharing syringes or other sharing equipment remained disappointingly high (80.3%). The vast majority of IDUs were men (82.5%) with a mean age of 33.7 years old.

The prevalence of hepatitis C in IDUs in Greece is extremely high (EKTEPN report 2006). The high HCV infection rates in Greek IDUs in the 5-year period 2001-2005(43.3-61.7%) appears to remain stable. The 2006 data are in line with the data from previous years, confirming that HCV infection rates normally increase with age and with the number of years of injecting use.

According to our data, 9/10 HCV infections acquired after 1992 belong to the IDUs group. Furthermore, one out of two IDUs were younger than 20 years when they acquired the infection. As a consequence, these findings show that in the very near future we will be facing a considerable number of young patients with cirrhosis, liver failure and HCC. The promising response rates to antiviral treatment in younger patients with HCV genotype 3 infection, suggests accessible HCV treatment programs for all IDU's. The finding that IDU is the dominant mode of HCV infection, is in agreement with data from other Western European countries[11,12] and underline the urgent need for universal prevention strategies in order to face the problem of drug addiction and HCV infection.

Interestingly, a relatively high prevalence (13.9%) of genotype 4, was observed in the present study. This rate was slightly higher than the one reported in a previous study conducted in Greece during 1987 to 2002. In this study, a phylogenetic comparison of the Greek 4a isolates with all HCV-4a isolates reported worldwide revealed a topology which does not discriminate Greek isolates from others. This finding suggests that HCV-4 does not represent a recent introduction in Greece[13] although there was a massive repatriation of Greeks from Egypt during the 60s. In our study we found that among immigrants, those infected with HCV genotype 4 were mainly from Egypt. However, immigrants represent only a small proportion of our sample, including those with genotype 4. A low prevalence of genotype 4 has been reported in Eu-

rope and USA[14,15]. As a result, Greece seems to have one of the highest incidence of genotype 4 HCV infections among European countries.

Further remarkable results of our study include the low percentage of subjects with antiviral therapy history and the late presentation at the hepatology centers (about 12% of the patients had advanced liver disease at their first visit and only 7.2% of the patients had a treatment history).These findings point out the urgent need to set-up awareness programs in order to sensitize the population.

There are, however, some limitations in our study: a) our study population may not be representative of the general Greek patients' population. The fact that most of the large hepatology centers (9 in Academic and 11 in Community hospitals) from the whole of Greece participate in the HEPNET.GREECE study makes this less likely. b) The retrospective design of the current study may have led to selection bias. The large number of participating patients, the inclusion of all patients followed-up (i.e. at least 2 visits) in the participating centers during the study period regardless of their treatment or final clinical outcome, the exclusion of patients with missing crucial data and the quality control of our data may partly overcome the limitation of the design. c) Possible route of transmission was unknown for a substantial number of the study population. Our analysis revealed that the demographic and clinical profile of those patients was similar to that of patients infected through blood transfusion. Therefore, most likely, these patients represent old infections. However, taking into consideration that their date of infection is unknown, this hypothesis cannot be tested. d) Noteworthy, from June 2003 the cohort has been followed-up prospectively and patients visited the participating clinics after this date were also included. One could argue that the fact that our study includes both retrospective and prospective information may have added bias. However, comparing the basic demographic characteristics (the possible route of infection was included) of our patients by cohort type (retrospective vs prospective) showed no significant differences in the percentage of missing data (data not shown).

In conclusion, our results show that the epidemiology of HCV infection is changing in Greece, with a substantial increase of genotype 3 and a reduction of genotype 1 probably due to the increase of IDU's. Furthermore, due to the rising prevalence of HCV among "new IDUs", those risk behaviors should become the focus of interventions for the prevention of infectious diseases in the drug user populations. At the same time, there is a need for more systematic treatment of hepatitis C in the drug users. However, although IDUs represent the majority of cases of HCV infection, many are excluded from receiving treatment because of concerns about adherence to treatment, the postulated high risk of re-infection and an increased risk of interferon-mediated neuropsychiatric side effects. Nonetheless, the rationale for this exclusion are often not based on suitable prospective and controlled clinical studies and our observations strongly support the clinician's standpoint to initiate treatment in order to reduce not only the burden of disease but also the high cost of managing patients with advanced disease.

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