

HEPATITIS C VIRUS GENOTYPES IN A COHORT OF MIDDLE EASTERN PATIENTS

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Background: The epidemiology of hepatitis C virus infection has been well characterized in Western Europe, North America and Japan. Less is known about it in other regions of the world. In order to fully understand the relationship between host and virus, it is important to study the effect of virus infection in all regions of the world. In this report, we have analyzed patients from the United Arab Emirates, Egypt and Jordan.

Design and Methods: Serum from 81 Middle Eastern HCV ELISA-2-positive patients was analyzed for the presence of HCV RNA by PCR. RNA-positive patients were genotyped by selective hybridization of amplicons to HCV genotype-specific oligonucleotides (InnoLipa2, Innogenetics, Belgium). Where possible, data was also obtained on racial origin, liver histology, serum ALT, prothrombin time, albumin, and risk factors for infection.

Results: Sixty-five of 81 patients were HCV RNA-positive. A higher proportion of Middle Eastern patients were genotype 4 compared to equivalent studies from Western Europe, USA and Japan. However, the most common genotype was 1a. No significant difference in genotype was found between patients with chronic hepatitis and patients with cirrhosis.

Conclusions: Eight of 65 (12%) patients were genotype 4, but the most common genotype was 1a, a "Western" genotype (24/65, 37%). The mean age of cirrhotics was low compared to Western studies. This may be due to infection in early childhood or race-related host factors. Twelve of 65 patients (18%) were not classifiable for genotype using InnoLipa2. This may be due to multiple infecting genotypes in these patients, or unusual, non 1-3 HCV genotypes which cannot be classified by InnoLipa2.

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It is not clear why chronic hepatitis C virus infection is associated with a broad spectrum of liver diseases. Patients can present with everything from asymptomatic mild chronic hepatitis to hepatocellular carcinoma.^{1,2} One possible explanation for the different manifestations of hepatitis C virus-related disease in different patients is that different strains of the virus have different pathogenetic effects on the human host. Numerous hepatitis C virus isolates from around the world have been cloned and sequenced, and it has become apparent that there is a high degree of sequence variation in some regions of the genome.³ From this data the hepatitis C virus has been divided into different genotypes based on sequence variability.

A total of nine major genotypes and at least 30 subtypes have been identified to date.³⁻⁶ The nomenclature proposed by Simmonds et al.⁷ has been widely adopted. This reflects a two-tiered hierarchical distribution based on phylo-

genetic analysis, which represents the mean evolutionary distance between different hepatitis C virus sequences. Hepatitis C virus genotypes are designated by Arabic numerals in order of discovery, and hepatitis C virus subtypes are designated using these numerals followed by lower case letters, also in order of discovery.

The clinical consequences of hepatitis C virus sequence variability are not yet clearly defined, but it has been reported from France, Italy and the United Kingdom that hepatitis C virus genotype 1, particularly 1b, is associated with more severe liver disease.⁸⁻¹² One potentially confounding factor in the studies from Pozzato et al. and Qu et al. is that the mean age of the patients with genotype 1b was significantly higher than the patients infected with other genotypes, thus the severity of liver disease could be related to duration of infection rather than genotype.^{10,11} However, the case for a direct pathogenetic effect of virus genotype is strengthened by data suggesting that patients with genotype 1b infection develop acute and/or chronic hepatitis following liver transplantation.¹³ Also, infection with genotype 1b predicts a poorer response to α -interferon in studies from Japan^{14,15} and France.^{11,12} Studies from Italy and the United Kingdom which did not subtype found that genotype 1 was less responsive to α -interferon.⁸

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In this report, we analyzed patients from the United Arab Emirates (UAE), Egypt and Jordan, areas where the epidemiology of hepatitis C virus infection has been less well characterized. Hepatitis C virus genotype was compared with liver disease severity by analysis of liver biopsy specimens and biochemical and hematological markers of liver disease. Other factors such as age and risk factors for hepatitis C virus infection were also studied in the same population to determine their influence on hepatitis C virus disease state.

Materials and Methods

Collection of Serum Samples

Serum samples from 81 Middle Eastern HCV ELISA-2-positive patients were collected in two hospitals in the UAE, and in patients from the Middle East referred to a tertiary referral center for liver disease in Newcastle-upon-Tyne (UK). No patients were resident in the United Kingdom and all had come from the Middle East for further medical opinion. Samples were centrifuged within two hours of collection and serum was aliquoted and stored at -20°C prior to transport to the Medical School in Newcastle. Serum was transported to Newcastle on dry ice and stored at -80°C until analysis. Patient details, including risk factors, age, duration of infection, racial origin, serum albumin level, serum prothrombin time, and liver biopsy histology, were obtained where possible from the case notes.

Hepatitis C Virus Genotyping

Each serum sample was analyzed for the presence of hepatitis C virus RNA by polymerase chain reaction (Amplicor HCV kit, Roche Diagnostic Systems, according to manufacturers' instructions). Three negative controls were run with each positive sample run. Strict protocols for minimizing the risk of contamination were observed according to the guidelines of Kwok and Higuchi.¹⁶ RNA-positive patients were genotyped by selective hybridization of amplicons to HCV genotype-specific oligonucleotides (Inno-Lipa2, Innogenetics, according to manufacturers' instructions). Samples were blinded at polymerase chain reaction level and genotyping level and only compared with source data after each sample run was completed.

Results

Hepatitis C Virus Genotypes and Liver Diseases

Sixty-five of 81 (80%) hepatitis C virus ELISA-2-positive patients were HCV RNA positive, and were genotyped by InnoLipa 2. The genotype distribution is shown in Figure 1. Thirteen HCV RNA-positive patients either had mixed genotype infection or were not classifiable using the key for genotyping supplied by the manufacturers, despite two attempts at genotyping each

serum specimen. The disease state was compared to hepatitis C virus genotype where data was available, by classifying patients with chronic hepatitis on liver biopsy and/or elevated ALT as liver disease, non-cirrhotic, and patients with cirrhosis on liver biopsy and/or prolonged prothrombin time and/or low serum albumin as cirrhotic. Using these parameters, clinical data was available on 65 patients. The hepatitis C virus genotype distribution and comparison with liver disease is shown in Table 1. No significant difference in genotype was found between non-cirrhotic and cirrhotic groups. Hepatitis C virus genotype was also analyzed with respect to racial origin. No significant difference in genotype was found between patients from Egypt, UAE and Jordan.

Age, Sex, Duration of Disease and Disease State

The male:female distribution was 64:17. There was no significant difference in disease state between males and females. Thirty-eight patients were non-cirrhotic, with a mean age of 41.9 ± 10.1 years and 27 patients were cirrhotic, with a mean age of 44.5 ± 9.9 years. This difference in age between cirrhotics and non-cirrhotics was not significant. Mean duration of disease was greater in cirrhotics (16.7 ± 7.5 years) than in non-cirrhotics (11.7 ± 7.2 years), but this was not significant due to the relatively high standard deviation.

Risk Factors

Risk factors identified from clinical data were transfusion (12), previous parenteral therapy for schistosomiasis or bilharzia (16), health care worker (2), intravenous drug abuse (2), and previous surgery (4). No significant difference in genotype was found between risk groups. Risk factors were also compared to disease state

TABLE 1. Hepatitis C virus genotype related to liver disease.

Genotypes	Non-cirrhotic	Cirrhotic	Total
1	2	3	5
1a	16	8	24
1b	1	2	3
2	0	1	1
3	0	3	3
4	6	2	8
Mixed	1	1	2
Unclassified	7	4	11
RNA negative	5	3	8

TABLE 2. Risk factors for hepatitis C virus infection compared to disease state.

Disease state	Risk factors				
	IV drugs	Health care worker	Transfusion	Previous parenteral therapy	Previous surgery
Non-cirrhotic	2	2	6	10	4
Cirrhotic	0	0	6	6	0

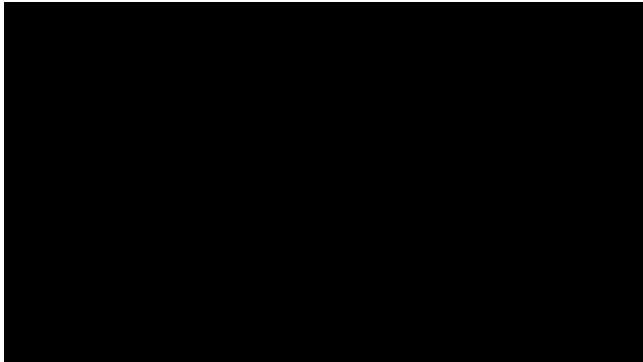


FIGURE 1. Distribution of hepatitis C virus genotype in the cohort of Middle Eastern patients studied. 1*=genotype 1, unclassifiable into 1a or 1b; M=mixed genotype; U=unclassifiable genotype.

(Table 2). Again, no significant difference in risk factors was identified between cirrhotic and non-cirrhotic patients.

Discussion

In this study, a higher proportion of Middle Eastern patients were classified as hepatitis C virus genotype 4 compared to equivalent studies from Western Europe, USA and Japan. However, the most common genotype was 1a, a "Western" genotype. Also, using the Inno-Lipa2 genotyping kit, 13 of 62 patients (21%) were either classified as mixed genotypes or were not classifiable for hepatitis C virus genotype. This may be due to multiple infecting genotypes in these patients, or unusual, non 1-3 HCV genotypes in this Middle Eastern patient group which cannot be classified by Inno-Lipa2.

No relationship was found between disease state, risk factors for infection, and genotype. Many patients in this study were unaware of the duration of infection and this may be a more important variable than genotype or risk factors in determining the progression of disease. In patients in whom the duration of infection was identified, there was a trend seen towards increased duration of infection in the cirrhotic patients. The mean age of cirrhotics was low (44.5 ± 9.9 years) compared to Western studies. A study previously carried out in the North East of England in a predominantly Caucasian population revealed a mean age in cirrhotics of 60.7 ± 12.5 years.¹⁷ This may be due to an increased rate of infection in early childhood in Middle Eastern populations, whereas the English population studied may have been more likely to acquire the disease in adult life.

The relative influence of hepatitis C virus genotype, risk factors for infection, duration of infection, and host factors on liver disease state are still not clear. Further study of infecting hepatitis C virus strains in non-Caucasian patient groups, who seem to progress to cirrhosis earlier in life, may help in determining the relative influence of hepatitis C virus genotype on the progression of liver disease.

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