Hepatitis C virus infection in Brunei Darussalam: A genotypic study

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Abstract

Hepatitis C virus (HCV) infection is one of the major causes of complicated liver disease. Viral genotypes have been shown to significantly affect treatment outcomes and may be a factor in disease progression. The aim of this study was to assess the genotypes in our local setting. Patients with HCV infection and had undergone genotypic testing were retrospectively reviewed. There were 75 patients (61 male, mean age 40.8 ± 9.8 years) who had genotypic testing. Four genotypes were detected; genotype 1 (n = 26, 34.7%), genotype 2 (n = 4, 5.3%), genotype 3 (n = 45, 60%) and genotype 4 (n = 2, 2.7%). Two patients were infected with dual genotypes (1 & 4 and 2 & 4 respectively). Both these patients had blood transfusion more than 20 years ago, one had transfusion in Egypt. The median viral load was 2.74 x 10^6 copies (range, 2,490 to 39 x 10^6). Between the favourable (genotypes 2 & 3) and non-favourable (genotypes 1 & 4) genotypes, there were no significant differences in the age ($p = 0.314$), between gender ($p = 0.226$), the mode of acquisition (intravenous drug injections vs. others, $p = 0.223$), viral load (< or ≥ 2 x 10^6 copies, $p = 0.763$) and the baseline serum alanine aminotransferase activity ($p = 0.403$). In conclusion, our study showed that the commonest genotype is genotype 3 followed by genotype 1. There were no significant differences between the favourable and non-favourable genotypes in the patients' demographics, mode of acquisition and necro-inflammatory activities and viral load.

Introduction

Chronic liver disease (CLD) is an important cause of morbidity and mortality. In the Asian Pacific region, Hepatitis B virus infection represents a major aetiology whereas in the United States, Europe and Japan, Hepatitis C virus (HCV) infection is the main aetiology [1, 2]. In the West and Japan, HCV has been estimated to account for 80% of chronic hepatitis, 40% of cirrhosis, 70% of hepatocellular carcinoma and 30% of transplant indications [3]. An estimated 150 to 200 million individuals are affected by this infection worldwide with 3 to 4 million new infections annually. HCV infection is now the leading cause of end stage liver disease and the leading indication for liver transplantation in Europe and North America. Overall, the incidence of HCV infection has decreased since its discovery in 1989. However, it is still common in a subset of the population, particularly among those in correctional and institutional facilities, intravenous drug users (IVDU), homeless, and patients with end stage renal failure (ESRF) undergoing haemodialysis [2, 4, 5].

HCV is a positive-stranded RNA virus of approximately 9,400 nucleotides that belong to the Flaviviridae family. HCV accounts for the majority of the non-A non-B chronic hepatitis [6]. Sequence analysis and comparisons of variants isolated from different geographical areas have led to the identifications and classifications of at least six genotypes (designated 1-6) [7]. Some of these genotypes contain a number of closely related, yet distinct subtypes of the virus (designated a, b, c, etc...). There are currently more than 50 subtypes [2]. These differences in nucleotides sequences identified may be as much as 20 to 23%.

Treatments for HCV infection are now better defined with the combination of interferon and ribavarin. Viral characteristics such as viral load and genotypes have been shown to be important factors that predict treatment outcome [2].

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Favourable genotypes were those shown to have favourable responses to standard therapy, consisting of mainly genotypes 2 and 3. Genotypes 1 and 4 have less favorable responses. There is currently limited information on the other remaining genotypes. Genotype has also shown to affect the natural history of HCV infection [8]. There are currently six distinct genotypes identified and different genotypes are more prevalent in different regions (Table 1) [9]. There is no published data regarding HCV infection in Brunei Darussalam. This study assesses the genotypic distributions among HCV infected patients, including the favourable and non-favourable genotypes, in patients in Brunei.

Table 1. Hepatitis C virus genotypes and their distributions

Genotypes	Distributions
1	Europe, North America, Japan
2	Southern Europe
3	Southeast Asia
4	Middle East, Egypt, Central Africa
5	South Africa
6	Indochina

Methods

Patients who were detected to have a positive HCV IgG serology are those that are routinely referred to the Hepatology Clinic for further evaluation including: HCV IgG, liver function test, detection of chronic liver disease, routine screening of blood donors, patients with ESRF undergoing haemodialysis and incarcerated inmates for suspected or confirmed drug offences. These patients were also routinely checked for co-infections particularly with hepatitis B virus or HIV virus. Intra-venous drug users (IVDU) were also checked for syphilis infection. Such patients were routinely followed up and evaluated for the need of treatment using the current standard pegylated interferon and ribavarin. Routine HCV RNA detection by PCR and genotyping with specific probes was performed through the contracting laboratories of the Ministry of Health (National University Hospital laboratory, Singapore and Gribbles Pathology, Kuala Lumpur and Melbourne).

Presences of risk factors (IVDU, haemodialysis or HD, previous operations, blood transfusion, other percutaneous procedures or positive sexual contacts) were regularly checked. Modes of HCV acquisitions were determined according to the risk factors. In patients with multiple risk factors, the most likely risk factor to be strongly associated with HCV transmission was considered to be the underlying aetiology.

Patients with HCV infection were identified from the clinics (Hepatology Clinic, RIPAS Hospital, Bandar Seri Begawan) and laboratory registries and retrospectively reviewed. Only patients followed in the Hepatology clinic in RIPAS hospital was included in the study. The Hepatology Clinics in RIPAS Hospital receive all referrals and follow up patients with chronic HCV infection. RIPAS Hospital serves three of the four districts with population catchments of approximately 320,000 (Economic Planning Unit, Ministry of Finance, projected population 2005). The other hospital, Suri Seri Begawan Hospital located in Kuala Belait, also sees and manages a smaller number of patients. The central laboratory handles all the blood testings for HCV and receives all the results for viral load and genotype testings.

At the time of the study (up till October 2005), there were a total of 185 patients under the follow up of the Hepatology Clinics and of this, 75 patients had undergone genotype testing. Blood samples were collected and send to the overseas centre for PCR and genotyping. Demographic data (age, gender and race), mode of acquisition (IVDU, transfusion related, haemodialysis related or others) and laboratory investigations (liver function test, viral load and viral genotype) were retrieved from case notes and computer results where available.

All data were coded and entered into the SPSS package (Version 10.0, Chicago, IL, USA) for analysis. The *Student's t-test, Chi-squared* and *Fisher's* exact tests were used when appropriate. The modes of acquisition were grouped into IVDU and others as in the comparison between favoruable (genotypes 2 and 3) and non-favourable (genotypes 1 and 4) genotypes. The results were considered to be statistically significant when p value is < 0.05 (2-tailed).

Results

The mean age of the patients was 40.8 ± 9.8 years old, consisted mainly male (81.3%) and predominantly Malay (80%). The most common mode of acquisition of HCV was through IVDU (63.5%). Two patients were co-infected with hepatitis B virus and none were positive for HIV infection. The demographics of patients and mode of acquisition of HCV infection are shown in Table 2.

Table 2: Baseline demographic data, mode of acquisition and baseline liver function test of patients with genotypic testings

Age (years)	40.8 ± 9.8
Gender	
Male	61 (81.3%)
Female	14 (18.7%)
Race	
Malay	60 (80%)
Chinese	8 (10.7%)
Indigenous	6 (8%)
Others	1 (1.3%)
Mode of acquisitions *	
Intravenous drug use	33 (63.5%)
Haemodialysis	6 (11.5%)
Transfusion/operation	6 (11.5%)
Sexual contact	3 (5.8%)
Unknown	7 (7.7%)
Baseline Liver function test	
Protein (gm/L)	79.4 ± 5.2
Albumin (gm/L)	39.0 ± 3.9
Bilirubin (mmol/L)	17.2 ± 8.9
Alkaline phosphatase (U/L)	78.7 ± 24.9
Gammaglutamyl transpeptidase (U/L)	68.7 ± 83.5
Alanine aminotransferase (U/L)	91.6 ± 63.9

* Based on 52 patients (23 patients did not have this data recorded)

There were four genotypes detected and the commonest genotypes were genotypes 3 and 1, accounting for 58% and 34% respectively. Genotype 2 accounted for 5%. Figure 1 show the distribution of genotypes. Two patients were infected with dual genotypes (1 & 4 and 2 & 4 respectively). Both these patients had blood transfusion more than 20 years ago, and one had it in Egypt.

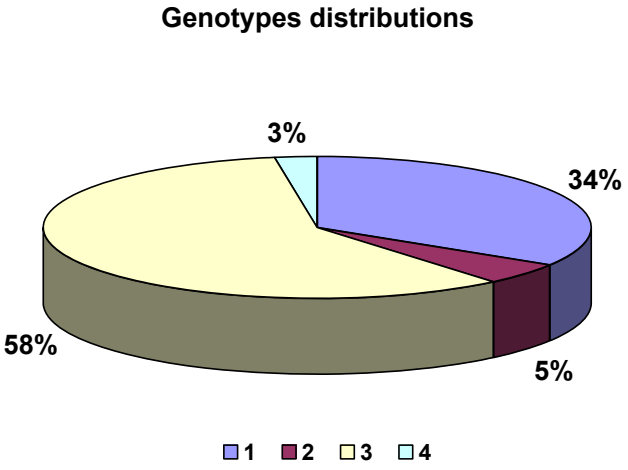

Figure 1. Genotypes distribution of hepatitis C virus

The median viral load was 2.74 x 10^6 copies (range, 2,490 to 39 x 10^6).

There were no significant differences between the favourable (genotypes 2 & 3) and non-favourable (genotypes 1 & 4) genotypes. Although those with favourable genotypes were younger, this was not statistically significant (p = 0.314). Similarly, there were no difference between genders (p = 0.226), the mode of acquisition (IVDU vs. others, p = 0.223), viral load (< or ≥ 2 x 10^6 copies, p = 0.763) and the baseline liver function test including the baseline serum alanine aminotransferase activity (p = 0.403), necroinflammatory and the fibrosis stages between the favourable and non-favourable genotypes. This is shown in Table 3.

Table 3. Comparison between patients with favourable (genotypes 2 and 3) and non-favourable (genotypes 1 and 4) hepatitis C virus genotypes

Parameters	Favourable	Non-Favourable	*p* value
Demographics			
Age (yrs)	39.9 ± 8.4	42.4 ± 11.8	0.314
Gender (male)	85.4%	74.1%	0.226
Mode of acquisition			
IVDU	69.7%	52.6%	0.223
Others	30.3%	47.4%	
Blood investigations			
Alanine aminotransferase (U/L)			
Baseline	96.8 ± 61.9	82.6 ± 67.5	0.403
Highest level	117 ± 70.9	118 ± 116.2	0.967
Albumin (gm/L)	39.3 ± 3.9	38.6 ± 4.3	0.633
Alkaline phosphatase (U/L)	73.5 ± 25.4	87.6 ± 22.2	0.107
Gammaglutamyl transpeptidase (U/L)	61.2 ± 37.2	82.5 ± 133.9	0.466
Protein (gm/L)	79.5 ± 5.4	79.2 ± 4.9	0.832
Viral load (≥ 2 x 106 copies)	48.4%	52.9%	0.763
Overall Cirrhosis	0.0%	11.1%	0.180
Histology			
Grade of inflammation	1.6 ± 1.0	2.0 ± 0.7	0.478
Stage of fibrosis	1.8 ± 0.9	1.4 ± 0.9	0.450

IVDU: Intravenous drug users

Discussion

Our study showed that four genotypes exist in Brunei Darussalam with the commonest genotype being genotype 3. Genotype 2, the other favourable genotype accounted for only 5%. Overall, favourable genotypes accounted for 63%. This finding is consistent with published data. These genotypes have been shown to have more favourable responses to treatment. Genotypes 1, 2 and 3 have a worldwide distribution with type 1 (especially subtypes a and b) being commonest, accounting for about 60% of global infections. In our setting, genotype 1 accounted for a third of our cases. Interestingly, we also found two patients with genotype 4, most probably acquired outside of the country. This genotype is uncommon outside of the Middle East, Egypt and Central Africa. Both of our patients had co-infections with other genotypes (1 and 2 respectively) and one had a history of blood transfusion more than twenty years before in Egypt. The other patient only had a history of blood transfusion more than twenty years ago. Whether both genotypes were acquired simultaneously is possible. However it is more likely than the different genotypes were acquired at different times. Currently it is not known whether co-infections with multiple genotypes have any impact on the natural progression. However, treatment is usually targeted at the less favourable genotype. We did not find any genotypes 5 and 6 among our patients. This is an expected finding as these genotypes are found only in Indochina and South Africa.

Reports from Europe and North America show that co-infections of HCV with other viruses, especially with HIV are not uncommon [10, 11]. This is particularly true in those with history of IVDU [12]. Similarly, co infections with HBV also occur [13, 14]. Currently, there are very little data from the South East Asia regions. Fortunately, in our setting, the incidence of HIV infections remains low and none of our patients had HIV infection. Only two had co-infections with HBV. Co infections with HBV can be managed concurrently as interferon used for HCV treatment is also a standard treatment for HBV.

Studies have shown that genotypes may influence the natural history and be associated with disease progression. Genotype 1, especially subtype b has been shown to be associated with higher risk of hepatic decompensation by three fold in patient with established liver cirrhosis [8]. Presence of steatosis may be associated with disease progression [15] and genotype 3 has been shown to be strongly associated with hepatic steatosis. However this is reversible with successful treatment. [16] Unfortunately, we were not able to assess this correlation as the number of patients having had liver biopsies was too small. However, there no significant differences when we compared between the favourable and unfavourable genotypes in the patients' characteristics, necro-inflammatory activities and viral load.

The commonest mode of acquisition among our patients was through IVDU followed by HD. With the implementation and improvement of infection control measures (designated HD machines and points), infection through HD is becoming less and IVDU is now becoming a major source of HCV infection in our local setting. A local study looking at patients with HCV undergoing HD in Suri Seri Begawan Hospital showed that the infection rate had declined significantly through these infection control measures [17]. Among those patients who acquired HCV after starting HD, the median time for HCV IgG to become positive was 45 months (range, 20 to 70). It is more difficult to estimate time of infection among IVDU as most were detected later once they were incarcerated. Furthermore, most were not very forth coming or cannot remember the exact time of starting IVDU. Furthermore, time of HCV acquisition is not necessary correlated to the first time of IVDU use. Among the different modes of acquisition, the favourable genotypes were more common among our IVDU group; however this did not reach statistical significance. This is important as this has cost saving as treatment duration for favourable genotypes is shorter.

Current treatment guidelines recommend six month of therapy for favourable genotypes (genotypes 2 and 3) regardless of viral loads and non-favourable genotypes with low viral load ($< 2 \times 10^6$ copies). However, 12 months of therapy have been recommended for non-favourable genotypes with high viral load ($\geq 2 \times 10^6$ copies). [2] More importantly, there are studies now showing that even shorter duration of treatment may be adequate for the favourable genotypes. [18, 19] Success rate in term of sustained viral response (absence of RNA detected six months post completion of treatment) have been shown to be as high as 76 to 82% for genotypes 2 and 3 compared to 42 to 46% for genotype 1 [2] Overall, this has cost implications as the treatment is costly and associated with significant side effects that can severely impair quality of life.

The main weaknesses of our study are the small sample size of patients having been tested for genotype and being a single centre study. This is mainly because at the time of the study, a proportion of patients already had been treated and some patients were still waiting for their results. Results from single center study may not be generalisable due to referral biases of tertiary centers. However, in our case, our clinics have a large population catchment of more than 75% of the overall population. Hence our results obtained are quite generalisable in our local contact.

In conclusion, our study showed that the commonest genotype is genotype 3 which is a favourable genotype. However, non-favourable genotypes are also common in particular genotype 1. Co infections are fortunately uncommon. There were no significant differences between the favourable and non-favourable genotypes in the patients' demographics, mode of acquisition, necro-inflammatory activities and viral load.

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