Epidemiol. Infect. (2007), **135**, 427–432. © 2006 Cambridge University Press
doi:10.1017/S0950268806006911 Printed in the United Kingdom

Genotypes of hepatitis C virus (HCV) among positive Lebanese patients: comparison of data with that from other Middle Eastern countries

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(*Accepted 29 April 2006; first published online 20 July 2006*)

SUMMARY

Recently we identified hepatitis C virus (HCV) genotype 4 as the principle genotype among Lebanese thalassaemics. In an attempt to confirm the predominance of genotype 4 in Lebanon and perhaps in the Middle East, genotyping was attempted on 142 HCV-infected Lebanese patients from five different hospitals in the country. These included 38 HCV-positive patients with symptomatic liver disease who were referred to gastroenterologists and 104 HCV-positive patients with no symptoms of liver disease: 27 patients with thalassaemia, 30 patients on haemodialysis, 32 multi-transfused and 15 intravenous drug users. HCV genotyping was performed on PCR HCV RNA-positive samples using a commercial line probe assay (LiPA; Innogenetics, Ghent, Belgium). HCV genotype 4 is found to be the predominant genotype among HCV-infected Lebanese patients (ranging from 34·2% to 53·3%) followed by 1a (ranging from 12·5% to 43·3%) and 1b (ranging from 8·0% to 34·4%). In patients with symptomatic liver disease, however, genotype 4 (34·2%) was preceded by genotype 1a (39·5%). The predominance of HCV genotype 4 in our population (45·7%) confirms the predominance of HCV genotype 4 in Lebanon and most of the Arab countries in the Middle East but contrasts with data reported from non-Arab Middle Eastern Countries as can be seen from the literature review. Implications of genotyping for clinical outcome of HCV infection, response to treatment as well as for vaccine development are discussed.

INTRODUCTION

It is well established now that hepatitis C virus (HCV) is the leading cause of bloodborne non-A, non-B hepatitis worldwide [1]. The majority of HCV isolates studied so far can be divided into six major groups designated genotypes 1–6, with subdivisions in each (subtype a, b, c, etc.) [2–4]. Three additional genotypes (genotypes 7–9) have been proposed based on partial sequences at the 5′- and 3′-ends of the genomes of isolates from Vietnam and Thailand [5, 6] and recently another two (genotypes 10a and 11a) have been suggested based on sequences of isolates from Indonesia [7]. Genotypes 10a and 11a are new known as 3 k and 6 g respectively while genotypes 7–9 now

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fall into the genotype 6 group. Some genotypes (1a, 1b, 2a, 2b, 3a) are widely distributed around the world [8, 9], while others have a more restricted distribution. Genotype 4 is predominant in the Middle East (particularly Egypt), Zaire and Burundi [10–12], while genotype 5 has so far been mainly found in South Africa [13]. Genotype 6 on the other hand has only been found in Hong Kong, Macau, and neighbouring regions in South-East Asia such as Vietnam [5, 6]. There is increasing evidence that the HCV genotype with which an individual is infected may have important implications for the clinical outcome of infection and its response to interferon treatment [14–16]. Thus, typing of HCV isolates becomes an additional tool in the diagnosis and treatment of HCV infection [17].

In a recent population-based survey on hepatitis in Lebanon [18], a low prevalence of antibody to HCV (anti-HCV) (0·7%) was found in the Lebanese population. Similar anti-HCV prevalence rates (ranging from 0·4% to 0·6%) have been reported on Lebanese blood donors and health workers [19, 20]. Studies on genotyping of HCV in Lebanon, however, are scarce and involve very small numbers [21]. Recently, we have identified genotype 4 as the principal genotype (37%) among Lebanese thalassaemics followed by 1a and 3a (21% each) [22]. In order to confirm the predominance of genotype 4 in Lebanon, we attempted in this study to investigate the distribution of the HCV genotype in different groups of HCV-positive Lebanese patients taken from different hospitals across the country. This included patients with symptoms of liver disease who were referred to gastroenterologists and patients such as thalassaemics, patients on haemodialysis, multi-transfused and intravenous drug users (IDUs) who had no symptomatic liver disease. Our results are compared with those from other Middle Eastern countries.

METHODS

Patients and serum samples

The study involved 142 Lebanese patients who were positive for anti-HCV by ELISA. These included 38 patients who had symptomatic liver disease and were referred to gastroenterologists (27 males, 11 females; mean age 50·6 years), and 104 HCV-positive patients who were at risk of acquiring HCV infection but had no symptoms of liver disease. These patients included 27 patients with β-thalassaemia major or intermedia (20 males, 7 females; mean age 20 years), 30 patients on haemodialysis (17 males, 13 females; mean age 45·1 years), 32 multi-transfused patients (17 males, 15 females; mean age 53·3 years), and 15 IDUs (11 males, 4 females; mean age 24·4 years). Thalassaemics were from the Chronic Care Center, Hazmieh, while the other high-risk group patients were from Hotel Dieu de France Hospital, Beirut, St. Joseph Hospital in Beirut, and the Benevolent Islamic Hospital in Tripoli. Patients with symptoms of liver disease were from the American University of Beirut Medical Center referred from various parts of the country. A blood sample was collected during 2002–2003 from each of these patients during one of their regular follow-up visits. Sera were separated from the samples and stored at $-70\ ^{\circ}C$, in 100-μl aliquots, until further processing. Patients with drug- or alcohol-induced liver disease, autoimmune disease, or HBsAg positivity were excluded from the study.

Testing for anti-HCV and HCV RNA

Testing for anti-HCV was repeated in all recruited anti-HCV-positive patients using at least two of three commercial tests: a standard ELISA (bioMérieux bv Boseind, Boxtel, The Netherlands); a third-generation immunoassay that allows the detection of antibodies to the NS3, NS4 and NS5 core antigens of the virus (United Biomedical Inc., Hauppauge, NY, USA); and, if either or both of these assays gave a positive result, a line immunoassay (INNO-LIA HCV Ab III; Innogenetics, Ghent, Belgium) was performed. Only samples found positive in the line immunoassay were considered positive for anti-HCV. Detection of HCV RNA was attempted on a 200-μl sample of each serum found positive for anti-HCV, using a commercial, PCR-based test (Amplicor HCV test; Hoffman–La Roche, Nutley, NJ, USA). The manufacturer's instructions were followed and the internal control supplied by the manufacturer was added to each specimen, as an extraction and amplification control.

HCV genotyping

HCV genotyping was performed using a commercial line probe assay (LiPA; Innogenetics), according to the manufacturer's instructions. In this assay, a PCR product obtained by amplification of the 5′ non-translated region of the HCV genome was labelled with biotin, reverse-hybridized with 21 different probes, fixed on a nitrocellulose membrane and then

Table 1. *Hepatitis C virus (HCV) genotype distribution in Lebanon*

	HCV genotype						
Category of patients	1a	1b	2a	2b	3a	4	5
Patients with symptomatic liver disease ($n=38$)	15 (39·5%)	3 (8·0%)	4 (10·5%)	2 (5·3%)		13 (34·2%)	1 (2·6%)
High-risk groups							
Thalassaemics* ($n=27$)	4 (14·8%)	5 (18·5%)		1 (3·7%)	4 (14·8%)	13 (48·1%)	
Haemodialysis ($n=30$)	13 (43·3%)				2 (6·7%)	15 (50·0%)	
Multi-transfused ($n=32$)	4 (12·5%)	11 (34·4%)			1 (3·1%)	16 (50·0%)	
Intravenous drug users ($n=15$)		5 (33·3%)			2 (13·3%)	8 (53·3%)	
Total ($n=142$)	36 (25·3%)	24 (16·9%)	4 (2·8%)	3 (2·1%)	11 (7·7%)	65 (45·7%)	1 (0·7%)

* Nineteen out of the 27 thalassaemics were genotyped and reported earlier [18].
$P<0·001$ for the difference in genotype distribution between patients from the high-risk groups and those with symptomatic liver disease.

revealed using a streptavidin–phosphatase conjugate; the hybridization pattern produced not only reveals the presence of any of the six major HCV genotypes, but also allows for identification of the subtype.

Statistical analysis

The χ^2 test was used to determine the variation in genotype distribution between the different groups. Statistical significance was considered as $P<0·05$. All statistical tests were performed using SPSS statistical software package version 11 (SPSS Inc., Chicago, IL, USA).

RESULTS

HCV genotype distribution in Lebanon

HCV genotype distribution in 142 HCV-positive Lebanese patients is shown in Table 1. Overall, HCV genotype 4 was the predominant genotype (45·7%) followed by genotype 1a (25·3%) and 1b (16·9%). Genotype 4 was also the predominant genotype in the high-risk groups (48·1%–53·3%) but not in those with symptomatic liver disease (34·2%) where genotype 1a predominates (39·5%). Differences in genotype distribution were statistically significant.

HCV genotypes among examined positive cases in different Middle Eastern countries

HCV genotype distribution among HCV-positive Lebanese patients is similar to that in other Middle Eastern Arab countries except for Jordan where genotype 1a predominates (Table 2). In Middle Eastern non-Arab countries (Iran, Israel and Turkey) genotype 4 was minimally detected and genotypes 1a or 1b were the predominant genotypes.

DISCUSSION

To our knowledge, this is the first study to report on the genotyping of HCV in different groups of HCV-positive patients across Lebanon, representing close to a population-based sample. Our results show that HCV genotype 4 is the predominant genotype (ranging from 34·2% to 53·3%) followed by 1a (ranging from 12·5% to 43·3%) and 1b (ranging from 8·0% to 34·4%). Subtype 2a was detected only in patients with symptomatic liver disease (10·5%) while subtype 2b was detected in patients with symptomatic liver disease (5·3%) and in thalassaemics (3·7%). In symptomatic liver disease patients genotype 4 was not the predominant genotype (34·2%) and was preceded by subtype 1a (39·5%). This difference in genotype distribution can perhaps be explained in light of the finding that genotype influences clinical outcome [23, 24]. Zekri *et al.* [25] showed that infection with genotypes 1a and 4 may be considered a risk factor for the induction of neu-oncoprotein overexpression and subsequent development of hepatocellular carcinoma (HCC). Amoroso *et al.* [26] showed the rate of evolution to chronicity after exposure to HCV was 92% in patients exposed to HCV genotype 1b infection compared with 33–50% in patients exposed to other genotypes. On the other hand, Zein *et al.* [27] found that patients infected with HCV genotype 1b were older than those infected with other genotypes and may have been infected for a longer period.

https://doi.org/10.1017/S0950268806006911 Published online by Cambridge University Press

Table 2. *Hepatitis C virus genotypes among examined positive cases in different Middle Eastern countries (expressed as %)*

Country	Category of patients*	Genotype									Comments
		1a	1b	2a	2b	3a	4	5	6	10a	
Lebanon ($n=142$)	SLD, HD, Th, MT, IDU	25·3	16·9	2·8	2·1	7·7	45·7	0·7			
Egypt ($n=89$)	ASC, CAH, HCC	12·4	2·2	11·2			57·3			1·1	15·7 mixed
Jordan ($n=30$)	HD	40·0	33·3				26·6				
Saudi Arabia ($n=119$)	CAH	10·1	16·8	0·8	1·7	1·7	48				16·8 not typable, 4·2% mixed
($n=353$)	CAH	5·0	16·0		0·6	5·9	62	0·3		0·3	
Syria ($n=37$)	HD	19	27				30				25% mixed 24% not typable
Iran ($n=15$)	CAH or cirrhosis	47	20			27	7				
Israel ($n=12$)	HD ($n=11$)										
	CAPD ($n=1$)	16·7	75			8·3					
Turkey ($n=36$)	HD	22·2	77·8								

ASC, Asymptomatic carriers; CAH, chronic active hepatitis; HCC, hepatocellular carcinoma; HD, haemadialysis; SLD, symptomatic liver disease; Th, thalassaemia; MT, multi-transfused; IDU, intravenous drug users; CAPD: continuous ambulatory peritoneal dialysis.
References: Data from Egypt [27], Jordan [28], Saudi Arabia [29, 30], Syria [31], Iran [32], Israel [33], Turkey [34].

Unfortunately we are unable to comment on genotype-clinical outcome relationship as liver biopsy was not performed on all our patients. Moreover, the date of initial exposure to HCV was unknown by us in most cases. The issue of the pathogenicity of different genotypes/subtypes remains controversial and long-term prospective studies in various population groups are required.

The predominance of HCV genotype 4 in the Lebanese population (45·7%) is in agreement with other reports on genotyping of HCV isolates in different Middle Eastern countries. Table 2 summarizes only the studies with relatively large numbers investigated [25, 27, 28–31]. It is of interest to note that in contrast to Arab countries genotype 4 can hardly be detected in non-Arab Middle Eastern countries such as Iran [32], Israel [33] and Turkey [34]. The presence of other genotypes such as 2a, 2b and 5 among Lebanese patients can be attributed to many factors. These include the traditional history of the openness of Lebanon to the outside world where the Lebanese migrated extensively to other countries for work and returned. On the other hand expatriates from different nationalities resided in Lebanon for quite some time and participated in blood donation. It is of interest that genotype 1 was the predominant genotype in patients with symptomatic liver disease in our study. A larger study examining the HCV genotype in Lebanese patients with symptomatic liver disease is under way to corroborate this finding.

A major area of clinical application of HCV genotyping has been in the study of significance of types/subtypes in response to antiviral treatment of HCV infection with interferon and ribavirin. Our initial results are in agreement with those of others [35–37] where patients with cirrhosis caused by HCV type 4 show poor response to interferon but improved outcome with combination therapy of pegylated interferon and ribavirin. The efficacy of peginterferon α-2b in combination with ribavirin has been recently evaluated in a relatively large number of patients in Kuwait with chronic HCV genotype 4 (66 patients) and a good response has been reported [38]. More studies, however, are still required to ascertain the pathogenicity, natural history and treatment guidelines of HCV genotype 4.

According to the World Health Organization, 180 million individuals in the world are infected with HCV and this is a growing global problem. The development of an effective vaccine remains the ideal way to combat HCV infection. In addition to the implications for clinical outcome of infection, and for

treatment, genotyping of HCV also has major implications for HCV vaccine development. Recent data suggest that for a vaccine to be fully protective it should contain a range of different envelope proteins corresponding to the common genotypes in particular geographic regions. Vaccines for use in the Middle East should, therefore, not be based only on genotype 4 sequences; other genotypes such as 1a and 1b are also equally important. Finally, genotyping of HCV may be a useful epidemiological marker particularly in establishing suspected unconventional routes of HCV transmission such as vertical [39], intraspousal, or intrafamilial transmission [40].

ACKNOWLEDGEMENTS

This project was partially funded by Schering-Plough and by the Lebanese National Council for Scientific Research. Special thanks go to the staff and the patients of the hospitals involved for their excellent cooperation and to Ms. Maha Abul Naja for her excellent secretarial assistance. The authors acknowledge the review suggestions of Professor Ross Brownson, Faculty of Public Health, St. Louis University, St. Louis, MO, USA.

DECLARATION OF INTEREST

None.

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