

Genotyping of Hepatitis C Virus (HCV) in Infected Patients from Yemen

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ABSTRACT

Hepatitis C virus has been considered to be one of the most important devastating causes of chronic hepatitis, cirrhosis, and hepatic cellular carcinoma. In this area, the epidemiology of hepatitis C is not well understandable in spite it was found to be endemic in Yemen. The distribution of hepatitis C virus (HCV) genotypes in the Yemen is unknown, so a molecular study was carried to investigate the prevalence of Hepatitis C virus (HCV) genotypes in HCV infected population of Sana'a, Yemen. A total of 502 HCV-positive clinical specimens (serum, EDTA plasma) with viral loads above 2,000 IU/ml were collected for genotyping. Genotyping of 502 samples revealed four different genotypes including 1 (1a and 1b), 2a, 3 (3a) and 4. The most prevalent genotype was 4 with rate of 63.7% followed by genotype 1a+1b (26.9%), 2a (7.6%) and 3a (1.8%). Genotypes 5 and 6 were not found in our patients. The predominance of HCV genotype 4 in our population confirms the predominance of HCV genotype 4 in Yemen and similar to most of the Arab countries in the Middle East.

Key Words: Genotypes, Hepatitis C virus (HCV), Sana'a, Yemen

Yemendeki Enfekte Hastalarda Hepatit C virüs (HCV) Gen Tiplemesi

ÖZET

Kronik hepatit, siroz ve hepatoselüler karsinomun en önemli tıhrip edici nedenlerinden birinin Hepatit C virüsü olduğu düşünülmektedir. Yemen'de endemik olarak bulunmasına rağmen, hepatit C'nin epidemiyolojisi bu bölgede iyi anlaşılmamıştır. Yemen'de hepatit C virüsü (HCV) genotip dağılımı bilinmemiyor, bu yüzden Yemen'in Sana kentindeki HCV ile enfekte nüfusta Hepatit C virüsü (HCV) genotiplerinin prevalansını araştırmak amacıyla, bu moleküler çalışma gerçekleştirilmiştir. Viral yükleri 2,000 IU/ml'nin üzerinde olan, toplam 502-HCV pozitif klinik örnek (serum, EDTA plazma) gen tiplemesi için toplanmıştır. 502 numunenin, dört farklı gen tipini de [1 (1a ve 1b), 2a, 3 (3a) ve 4] içeren gen tiplemesi saptandı. En yaygın genotip 4 (% 63.7) olup, bunu 1a +1 b (% 26.9), 2a (% 7.6) ve 3a (% 1.8) gen tipleri izlemektedir. Hastalarımızda 5 ve 6 gen tipleri bulunmadı. Popülasyonumuzdaki HCV genotip 4 baskınlığı,

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Yemen'deki HCV genotip 4'ün baskınlığını ve Ortadoğu'daki çoğu Arap ülkesindeki benzerliği doğrulamaktadır.

Anahtar kelimeler: Gen tiplemesi, Hepatit C virüsü (HCV), Sana, Yemen

INTRODUCTION

Hepatitis C virus (HCV) infection is a global public health problem. World Health Organization (WHO) estimates up to 3% of the world's population to be infected with HCV and there are approximately 180 million individuals are thought to be infected. On average, 80% of acutely infected individuals develop a chronic infection (1,2). HCV has a positive-sense single stranded RNA genome of flavivirus and about 9.6 kb containing one long open reading frame (ORF) with untranslated regions at both ends (3). So far, six major genotypes (HCV-1 to HCV-6) have been described, each containing multiple subtypes (for example, 1a, 1b, etc.) (4,5). The genotype of the HCV strain appears to be an important determinant of the severity and aggressiveness of liver infection, as well as patient response to antiviral therapy (6,7). HCV genotypes display significant differences in their global distribution and prevalence, making genotyping a useful method for determining the source of HCV transmission in an infected localized population (8,9). HCV genotype 1, 2 and 3 are distributed worldwide and their relative prevalence varies from one geographic area to another, whereas genotype 4 is predominantly prevalent in the Middle East and Africa, genotype 5 in South Africa and genotype 6 in South East Asia (5,6,10-12). According to several studies, genotype 4 is predominant in Egypt, Saudi Arabia, 4 and 1 in Kuwait and Syria, and genotype 1 in Lebanon, Iraq and Iran (13-17). Yemen shows an intermediate endemicity for HCV. Several studies from the end of 1990 and early 2000 found a low rate of endemicity for HCV, with 0.5% to 2% of blood donors from Sana'a and Aden and among an ethnically African group living in Sana'a (5%) and on the island of Socotra (5%) (18). By 2010, the prevalence of HCV infection in general population had declined to 1% or less (19). Blood donor results have also shown a steady decrease in HCV infection in which the prevalence declined from 5% in 2002 (18,20) to 1.3% in 2012 (19). Since HCV infection among children in Yemen is rare, inter-family and sexual transmission is very low, but one should consider other factors that could contribute to the continuing transmission of HCV among the Yemeni popu-

lation. Among the possible factors are blood transfusion, history of surgery and non-adherence to universal infection control precautions in medical services like hemodialysis in which high rate of HCV was reported (62.7%) (21) and dental clinics, sharing of needles and folk medical practices like cauterization and bloodletting (19,22). On the other hand HCV genotype prevalence rate and the role of this agent in acute and chronic liver disease and genotype response to treatment in Yemen is absent or at least poorly understood, so this study was carried out as the first study to detect the genotype prevalence rate of HCV and response to treatment in Yemen (published elsewhere).

MATERIALS AND METHODS

This was a laboratory descriptive study conducted from January 2008 to December 2012 from different hospitals as well as polyclinics at Al-Awalagy Medical laboratory, Sana'a, Yemen. The latter is a reference laboratory serving a number of hospitals and polyclinics in Sana'a. Serum samples were collected from 502 consecutive patients with varying grades of liver diseases, high alanine transferase (ALT) for >6 months, and positive HCV. Patients positive for anti-HCV antibodies were referred to the reference laboratory for detection of HCV RNA level and for genotyping. The study was designed to include patient's demographics (age, sex and locations) as well. Detection of HCV ribonucleic acid (RNA) was carried out on a 500 μ l sample of each sample positive for anti-HCV antibodies by both HCV enzyme immunoassay (EIA) and recombinant immunoblot assay (RIBA), using a commercial polymerase chain reaction (PCR)-based test (Taqman amplicor, Roche, USA) and following manufacturer's instructions. Internal control supplied by the manufacturer was added to each specimen, as an extraction and amplification control. HCV positive clinical specimens (serum, ethylenediaminetetraacetic acid) (EDTA) plasma with viral loads >2,000 IU/ml were selected for genotyping using Versant HCV genotype assay (LiPA) 2.0 (Innogenetics, Siemens Healthcare Diagnostics, USA). This kit allows an improved and more accurate distinction between HCV genotype 1 and subtypes c to l of genotype 6 as well as between subtypes a and b of genotype 1.

Data was analyzed using EPI Info -6 version. A p value of <0.5 was considered significant for statistical analysis.

Table 1. The age and sex distribution of hepatitis C virus patients, Yemen

Age groups	Male		Female		Total	
	No.	%	No.	%	No.	%
< 20 years	4	1.4	0	0	4	0.8
20 - 29 years	32	11	6	2.9	38	7.6
30 - 39 years	46	15.8	37	17.6	83	16.5
40 - 49 years	96	32.9	101	48	197	39
50 - 59 years	78	26.7	54	25.7	132	26.3
≥ 60 years	36	12.3	12	5.7	48	9.6
Total	292	58.2	210	41.8	502	100
Mean age	44.8 years		44.8 years		44.8 years	
S D	12.1 years		8.4 years		10.7 years	
Min	3 years		25 years		3 years	
Max	76 years		65 years		76 years	
Median	46 years		45 years		46 years	
Mode	50 years		45 years		45 years	

The study was approved by the Ethics Committee of our university and informed consent was obtained from all study participants.

among males (64.3%) and females (62.9%). Also distribution of types 1a and 1b was roughly similar among males (26%) and females (28.1%), while type 3a was more commonly seen among males (2.4%) ($p < 0.5$) (Table 2).

RESULTS

Table 1 shows the age and the sex distribution of our hepatitis C virus patients, their age ranged from 3 to 76 years, with the mean age \pm SD for total equal to 44.8 ± 10.7 years. Males counted 292 (58.2%) and females were 210 (41.8%). The genotype distribution in 502 HCV-positive patients is shown in Table 2. Overall, HCV genotype 4 was the most predominant genotype (63.7%) followed by genotype 1a and 1b (26.9%) and 3a (7.6%). Differences in genotype distribution were statistically significant ($p < 0.5$). Distribution of genotype 4 was roughly similar

DISCUSSION

Understanding of the geographic distribution of common genotypes requires detailed knowledge about the routes of transmission, prevalence of HCV in general population and in the various high risk groups, phylogenetic evolution of types and subtypes over a long period, and also for HCV medical treatment response, treatment duration and the dose of ribavirin (7,13). Unfortunately, there is little information available on these topics, particularly from Middle East countries and more specifically from Yemen.

Table 2. The prevalence of different HCV genotyping among our HCV patients, Yemen

Genotypes	Male n=292		Female n=210		Total n=502		P value
	NO	%	NO	%	NO	%	
Genotype 1a +1b	76	26	59	28.1	135	26.9	NS
Genotype 2a	21	7.2	17	8.1	38	7.6	NS
Genotype 3a	7	2.4	2	0.95	9	1.8	<0.05
Genotype 4	188	64.3	132	62.9	320	63.7	NS
Genotype 5	0	0	0	0	0	0	NS
Genotype 6	0	0	0	0	0	0	NS

NS=Non-significant >0.05

Studies suggest that infections caused by different HCV subtypes may have different clinical outcome and that some subtypes are associated more frequently with advanced liver disease and hepatocellular carcinoma (HCC) (23,24). This is the first published study to report on the genotyping of HCV in Yemen of HCV-positive patients.

The results show that HCV genotype 4 is the predominant genotype (63.7%) among Yemeni patients followed by 1a and 1b (26.9%) and 2a (7.6%), while genotype 3a was rarely found (1.8%). HCV genotype 4 is the predominant genotype among males and females. There is lack of comprehensive data on the distribution of HCV genotypes in Middle East countries, particularly in the Yemen; however there are few studies which have reported high prevalence of genotype 4 in these countries (15,16,25,26). Genotype 4 is also predominant in Egyptian patients as reported by Ray et al. (27) and Sievert et al. (5) as a most common genotype in Egypt.

The presence of other genotypes such as 1a and 4 among Yemeni patients can be attributed to many factors. These include the transfer of large number of Yemeni to foreign works and medication to Middle East countries in which, genotype 4 is predominant in Egypt, Saudi Arabia, 4 and 1 in Kuwait and Syria, and genotype 1 in Lebanon, Iraq and Iran (5,13-17,27).

The infection with genotypes 1a and 4 may be considered a risk factor for the induction of neuoncoprotein over expression and subsequent development of hepatocellular carcinoma (5,28). The presence of other genotypes such as 2a, and 1b among Yemeni patients can be attributed to many factors. These include the transfer of large number of Yemeni to Arabic neighbor countries and to other African and Asian countries and participated in contract infections from their.

The importance of HCV genotyping has considerably increased in the last few years. It has been used to study worldwide and local molecular epidemiology of HCV, and to trace sources of HCV infection in risk groups. Typing has also been used to study relationships between type/subtype and the clinical status, pathogenesis and/or outcome of disease. The major area of clinical application of HCV genotyping has been in the study of the significance of types/subtypes, in response to antiviral treatment of HCV infection with interferon and ribavirin, as well as the identification of patients with mixed infections. It has also been a useful application in vaccine research and development (24,29).

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