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Original Article

Prevalence of Mixed Hepatitis C Virus (HCV) Genotypes among Recently Diagnosed Dialysis Patients with HCV Infection

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ABSTRACT. Hepatitis C virus (HCV) infection is considered a major health problem recognized globally. HCV is a major cause of chronic liver disease that may lead to cirrhosis and hepatocellular carcinoma. The aim of this study was to investigate the prevalence of multiple (mixed) HCV genotypes in Saudi patients recently diagnosed with HCV infection and their association with various clinical risk factors. We examined a total of 1,292 newly diagnosed HCV-positive cases between January 2006 and July 2009 at the Molecular Pathology Laboratory, King Abdulaziz Medical City, Riyadh. The clinical and laboratory data of the study patients were collected. The HCV-RNA viral load and its genotyping were carried out with RT-PCR technology to assist in the follow-up and management of HCV-infected patients undergoing antiviral therapy. Twenty-two patients (1.7%) were found to have mixed HCV genotypes; of them, mixed genotypes associated with genotype-4 were seen in 19 patients (86%), mixed genotypes associated with genotype-1 were found in 68.4%, with genotype-3 in 26.3% and with genotype-2 in 5.3%. Additionally, mixed genotypes associated with genotype-1 were seen in three cases (13.6%); they were associated with genotype-2 in two (66.7%) and with genotype-5 in one patient (33.3%). In conclusion, the prevalence rate of mixed HCV genotypes in the cohort of the newly infected Saudi patients was 1.7%, with genotype-4 being the most frequent genotype encountered.

Introduction

Hepatitis C virus (HCV) infection is considered a major health problem recognized globally.[1] Worldwide, it is estimated that around 200 million people are infected with HCV, with an overall prevalence of 3.3% of the world's population.[2] Individuals infected with HCV are at risk of developing liver cirrhosis and hepatocellular carcinoma (HCC).[3]

The prevalence of HCV infection varies among blood donors worldwide, and is estimated to be between 1 and 5%.[2,4] HCV has six major genotypes according to its viral genome, numbered one to six. These viral types and sub-

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types differ in their geographical distribution and anti-genicity.[4] According to the World Health Organization (WHO) data, the highest HCV prevalence rates were reported in Africa, Eastern Mediterranean, South-East Asia and the Pacific regions compared with North American and European countries.[4,5] In Saudi Arabia, the prevalence of HCV among blood donors is estimated to be between 0.6 and 1%.[6] Worldwide, HCV infection in hemodialysis (HD) patients varies geographically, and both within and between countries, ranging from 3% in the USA to more than 70% in some other countries.[7-12] HCV infection in Saudi HD patients varies between different regions and provinces, ranging from 15% to 95%.[13] Genotype-4, followed by genotypes-1a and -1b, are predominant in Saudi patients, whereas genotypes-2, 3, 5 and 6 are rare.[13-16] Mixed infections (co-infection) with more than one genotype were observed in some studies. In this study, we investigated the prevalence of multiple (mixed) HCV genotypes in Saudi patients recently diagnosed with HCV, referred for genotyping to our institute.

Materials and Methods

Patients

Plasma samples from 1,292 newly diagnosed patients with HCV infection (HCV-RNA positive) who were referred to the molecular pathology laboratory were studied between January 2006 and July 2009. Two tubes of isolated EDTA plasma were processed within two hours of collection from each patient and stored at -80°C prior to performing the requested test.[17] HCV-RNA viral load and its genotypes were studied to assist in the management of the HCV-infected patient undergoing antiviral therapy. Before genotyping, detection of HCV RNA was mandatory to reconfirm the current HCV infection. Clinical and other laboratory data were collected on all patients.

HCV-RNA Viral Load Assay

A total HCV nucleic acid (RNA) was extracted from 1,050 µL of plasma from the patients and controls using the automated extraction Cobas AmpliPrep instrument (Roche Molecular Systems, Pleasanton, USA) and according to the manufacturer's instructions. HCV RNA amplification and detection were performed using the real-time Cobas TaqMan 48 analyzer (Roche Molecular Systems, Pleasanton, USA) manufacturer's recommendations. The final HCV viral load was reported in international units (IU)/mL.

HCV RNA Genotyping Assay

A 5 µL sample of the extracted RNA was added to 20 µL of each HCV Genotyping II kit master mix as per the manufacturer's instructions. Amplification and detection were performed using the m2000 real-time system (Abbott Molecular Diagnostics, Abbott Park, IL, USA).

Results

A total of 1,292 newly diagnosed HCV-positive cases, confirmed by HCV-RNA, between January 2006 and July 2009, were analyzed for genotyping prior to management. Only 22 (1.7%) cases of the 1,292 cases revealed presence of more than one HCV genotype (mixed). All the 22 cases were mainly associated with chronic hepatitis and other medical history, including age and sex distribution is shown in Table 1. Three cases were associated with chronic hepatitis and not related to any direct risk factors, although link to drug abuse was considered.

In 19 cases (86%), genotype-4 was seen to be associated with genotype-1, genotype-3 and genotype-2 (68.4%, 26.3% and 5.3%, respectively). Three cases (13.6%) of genotype-1 had associated genotype-2 and genotype-5 (66.7% and 33.3%, respectively). Presence of three mixed genotypes was seen in one case, and was associated with genotype-1, genotype-4 and genotype-5.

It was also important to note that mixed genotypes were more frequently seen in males (63.6%) as compared with females (36.5%).

Table 1. Summary of correlation between patient's clinical features and demographics with mixed HCV genotype.

No.	Age (years)	Gender	Mixed HCV genotype	HCV viral load*	Medical history					
					Dialysis	Transplant	Transfusion	Surgery	Liver disease	Other co-morbidity
1	48	F	3 and 4	389721	--	--	--	Yes	CH	Menorrhagia
2	52	F	3 and 4	102150	--	Liver	Yes	Yes	CH	DM
3	57	F	1b and 4	955	--	--	--	--	CH	DM, IHD
4	72	M	1 and 4	21544051	--	Liver	Yes		CH	--
5	52	M	1 and 2	13014939	Yes	Renal	Yes	Yes	CH	--
6	40	M	1 and 2	>500000	Yes	Renal	Yes	Yes	HB	--
7	68	M	1b and 4	4532909	--	--	--	--	CH	TB
8	53	M	3 and 4	23813		Liver	Yes	Yes	CH	--
9	30	M	1a and 4	3178058	Yes	Renal	Yes	Yes	CH	--
10	69	M	1b and 4	691000	--	--	Yes	--	CH, HCC	DM
11	56	M	1 and 4	174000	--	--		--	CH, HB	--
12	38	M	1 and 4	154000	--	--	--	--	Cirrhosis	--
13	67	F	3 and 4	637457	--	Liver	Yes	Yes	CH, HCC, cirrhosis	--
14	64	F	1 and 4	677344	--	--	--	--	Cirrhosis	Gastritis
15	66	M	1 and 3	609556	--	--	--	--	CH, HCC	--
16	60	M	1 and 4	30830					CH	--
17	53	F	3 and 4	1698844	Yes	Renal	Yes	Yes	CH	DM
18	68	M	1 and 4	3749058	--	--	--	--	CH	--
19	59	F	2 and 4	1730568	--	--	--	--	CH	--
20	32	M	1, 4 and 5	16988136	Yes	Renal	Yes	Yes	CH	--
21	52	M	1b and 4	4161073	--	--	--	--	CH	Alcoholic
22	59	F	1 and 4	2327460	--	--	--	--	CH	DM

CH: chronic hepatitis; HB: hepatitis B; DM: diabetes mellitus type-2; HCC: hepatocellular carcinoma, F: female; M: male; HCV: hepatitis C virus, IHD: ischemic heart disease, *Viral load at time of genotyping.

Discussion

It is well known that HCV is a major cause of chronic liver disease that may lead to liver cirrhosis and HCC.[3,18,19] In 1998, Poynard et al reported that between 20 and 30% of HCV cases will eventually progress to liver cirrhosis and, subsequently, to HCC.[20] This has been well documented in five of our patients (23%) who developed HCC or cirrhosis or both. According to our data, we anticipate that HCC and cirrhosis are more common among patients with co-existence of mixed HCV genotype.

Transmission of HCV is influenced by several risk factors, such as intravenous drug use, surgery, transplantation, dialysis, blood trans-fusion, liver diseases and diabetes mellitus.[1,2,5,7,12,16,19] Certain medications, chemicals, exposure to poisons and other toxins as well as alcohol abuse may influence the progress of HCV infection.[13] Only 19 cases (86%) with mixed HCV genotype were proven to be associated with various risk factors.

Three cases of mixed HCV genotype (13.6%) were shown to have chronic hepatitis, with the source of HCV transmission being unknown. The prevalence of HCV infection is increased in patients with type-2 diabetes mellitus,[12,21,22] and this was noticed in five of our patients (22.7%), compared with 21.2% in a study by Akbar in Saudi patients from the Western Province.[22]

Mixed HCV with more than one genotype was observed in some studies with higher prevalence rates in multiple-exposure groups such as hemophiliacs, patients on chronic HD and injection-drug users.[14,16,23,24] In this study, the prevalence of mixed HCV genotype was seen in 22 of the 1,292 patients (1.7%), compared with 5.3% in an Egyptian study,[23] 6% among Serbian patients,[24] 4% in Honk Kong and 18% in China.[25]

The prevalence rate of mixed HCV genotype in the cohort of newly infected Saudi patients was 1.7%, indicating the contribution and association of various risk factors to the clinical profile and disease progression in different patients.

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