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ORIGINAL ARTICLE

Distribution of Hepatitis C Virus Genotypes in Iranian Patients with Congenital Bleeding Disorders

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Abstract

Background: Chronic hepatitis C virus (HCV) infection is the major cause of liver disease related morbidity and mortality in hemophilic patients who needs regular blood product administration. Although genotype of infecting HCV is one of the prime predictors of response to antiviral therapy however, its distribution in hemophilic patients is still unclear and just few studies with low sample sizes have investigated this issue. Therefore, in this study, we aimed to identify this distribution in 367 Iranian hemophilic patients.

Methods: Blood samples were received from 367 hemophilic patients with chronic hepatitis C detected during a nationwide screening program who referred to our center for therapeutic measures. HCV RNA viral load was detected using Amplicor test (Version 2). Genotyping was performed by genotype specific primers.

Results: HCV genotype distribution was 1a in 58%, 3a in 18.5%, 1b in 14.7%, 4 in 1.1%, 2 in 0.8% and mixed in 6.2% and finally 0.5% of isolates were non-typable. Serum liver enzymes were not associated with HCV viral load and genotypes. Patients with severe bleeding tendency had significantly lower serum liver enzymes than those with a mild bleeding tendency.

Conclusion: Genotype 1a followed by 3a and 1b were the most frequently detected HCV genotypes in Iranian hemophilic patients and there was no association between splenomegaly and viral markers and liver enzymes in these patients.

Keywords: Hemophilia; HCV; Genotype; Congenital bleeding disorder; Iran

Introduction

Transfusion transmitted diseases are a major challenge to the health of patients with congenital bleeding disorders who need to receive regular blood products. Nowadays, Hepatitis C virus (HCV) infection is discussed as one of the most prevalent transfusion transmitted infections.[1-8] After the adoption of highly effective methods of viral inactivation in 1984-1987, HCV transmission substantially ceased among recipients of factor concentrates, however recent studies have demonstrated that prevalence of this infection is still a problem.[9-14] The seroprevalence in general population of Iran was reported to be 0.5-1 percent but rises to even 60 percent in hemophilic patients in different settings.[15-23] HCV infection in 60-80 percent of cases, institutes chronic hepatitis and impose the risk of cirrhosis, end stage liver disease and hepatocellular carcinoma, therefore its successful and timely treatment has a major value to quality of life of patients infected with this viral disease. Despite of HCV infected hemophilic patients in western countries like UK and USA that are at cirrhotic phase, in eastern countries like Iran, the majority of hemophilic patients have milder liver damage, therefore early diagnosis and treatment would significantly decrease the burden of liver diseases in the community. According

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to slogan,[24,25] of "start today, tomorrow may be late" with cooperation of Ministry of Health, Iranian Center for Disease Control and, Baqiyatallah Research Center for Gastroenterology and Liver Diseases, we launch the nationwide registering and screening program to detect hemophilic patients with HCV infection and starting treatment in order to decrease the burden of liver diseases related morbidity and mortality, rate of nasocomial and intra-familial transmission and improving their quality of life. This report is the primary result of our project, on hemophilic patients, aimed to identify genotype distribution of HCV in Iranian hemophilic patients.

Material and Methods

From a total 400 of hemophilic patients with clinically and laboratory proven chronic HCV infection, we enrolled 367 subjects in the study. Thirty three subjects (8%) refused to participate or were transferred to outreach centers. Informed consent has been also obtained from individual patients at the registration time.

The severity of bleeding disorders was designated as severe when levels of factor VIII or IX or the deficient factor activity were <1%; moderate when levels were between 1% and 5% and mild when levels were between 5% and 30%.

Virus RNA was extracted from 100 µL of serum mixed with 200 µL of 68% guanidine thiocyanate. To this solution, 200 µL isopropanol was added, then the sample was centrifuged for 15 min at 14000 g. The supernatant was removed and the precipitate was washed with 70% ethanol and then dissolved in 200 µL of distilled water treated with diethyl pyrocarbonate. Five µL of this preparation was added to a reagent mixture which contained 20 pmol of random hexamer, 200 U of Moloney murine leukemia virus reverse transcriptase, 8 unit of RNAse inhibitor and 10 nm of each deoxy nucleoside triphosphates, as well as 2 µL of 10x PCR buffer (50 mM Tris-HCL [PH. 8.2], 0.8 M KCL, 30 mM $MgCL_2$). The volume was adjusted to 20 µL and reactant was covered with 20 µL of mineral oil (Sigma Chemical Co., St. Louis, MO, USA) and was incubated at 42°C for 60 min. All polymerase chain reaction (PCR) procedures were performed as described previously.[26] The only difference was that for the second round PCR, 1 µL of the first run PCR product was added to nine different genotyping reaction tubes. Each tube contained primer S7 and only one of the genotype specific primers. PCR products were electrophorased on 3% agarose gel and stained by ethidium bromide. Genotypes were determined based on the molecular weight of each product as published before.[27] Viral load quantification was performed using the Cobas Amplicor HCV Monitor, V2.0 (Roche Diagnostics, Branchburg, NJ, USA) with lowest detection level of HCV RNA=50 IU/mL.

The statistical tests used were one-way ANOVA, t-student and Chi Square tests. All computations were carried out using SPSS software (version 16, Chicago, IL, USA). The probability value of $p<0.05$ was regarded statistically significant.

Results

Table 1 has summarized our subjects' baseline characteristics. Just 24 (6.5%) subjects were female. The mean age was 29-31 years. 256 (69.8%) subjects had hemophilia A. 156 (42.5%) subjects had serum HCV-RNA more than 1,000,000 copy/ml. Most of our subjects (61%) had also a severe bleeding disorder. Table 2 has listed the subtypes of genotypes in our subjects. The most frequent genotype was 1a (58%) followed by 3a (18.5%), 1b (14.7%), mixed genotype (6.2%), 4 (1.1%), 2 (0.8%) and 0.5% un-typable. There was not any association between presence of splenomegaly and infecting genotypes (Table 2). Liver biochemical profile and serum HCV viral load were not associated with infecting genotypes. Also there was not any association between the types and severity of bleeding disorder and HCV genotype (Table 2).

Discussion

Genotype is an important parameter used in selecting an antiviral therapy which fits the greatest chance of success. Genotyping and subtyping of HCV is relevant to the epidemiology of HCV, vaccine development, clinical management and assessment of the risk benefit ratio of therapeutic measures against chronic HCV infection.[28-35] It has been assumed that differences in nucleotide sequence of different genotypes could yield differential activity of HCV proteins resulting into a change in the rate of HCV replication, sensitivity to interferon, the antiviral activity of ribavirin or pathogenicity of the virus.[36] Substantial evidences have emerged indicating that typing and subtyping for HCV is important clinically; genotype 1

Table 1: Subjects' baseline characteristics among Iranian hemophilia patients with HCV infection

Patients' characteristics	***P-Value***
Sex	
Male, n (%)	343 (93.5)
Female, n (%)	24 (6.5)
Mean Age	30 (29-31)
BMI	23.6 (15-39)
Bleeding disorder type	
Hemophilia A, n (%)	256 (69.8)
Hemophilia B, n (%)	51 (13.9)
Von Willberand, n (%)	34 (9.3)
Others, n (%)	2 (0.5)
Deficiency Factor 2, n (%)	2 (0.5)
Deficiency Factor 5, n (%)	1 (0.3)
Bernard-sloiyer, n (%)	1 (0.3)
Glanzman syndrome, n (%)	10 (2.7)
Deficiency Factor 7, n (%)	2 (0.5)
Deficiency Factor 5, 8, n (%)	2 (0.5)
Deficiency Factor 10, n (%)	2 (0.5)
Deficiency factor 13, n (%)	4 (1.1)
Bleeding severity	
Mild, n (%)	30 (8.2)
Moderate, n (%)	70 (19.1)
Severe, n (%)	224 (61)
Unknown, n (%)	42 (11.4)
ALT U/L	64(56-72)
Normal (<40 U/L), n (%)	153 (41.7)
>2 folds increased, n (%)	69 (18.8)
AST U/L	43 (39-47)
Normal (<40 U/L), n (%)	223 (60.8)
>2 folds increased, n (%)	18 (4.9)
AST/ALT	0.81 (0.77-0.85)
>1, n (%)	73 (19.9)
ALP U/L	242 (227-256)
AFP ng/l	6 (4-9)
>10 ng/l, n (%)	20 (5.4)
Unavailable, n (%)	142 (38.7)
Log_{10} Serum HCV Viral Load copy/ml	5.88 (5.82-5.94)
>6, n (%)	156 (42.5)
Genotype	
Genotype 1a, n (%)	213 (58)
Genotype 3a, n (%)	68 (18.5)
Genotype 1b, n (%)	54 (14.7)
Mixed Genotype, n (%)	23 (6.2)
Genotype 4, n (%)	4 (1.1)
Genotype 2, n (%)	3 (0.8)
Untypable, n (%)	2 (0.5)
Splenomegaly	
Yes, n (%)	32 (8.7)
No, n (%)	262 (71.4)
Unavailable, n (%)	73 (19.3)

Table 2: Multiple comparisons between variables at enrollment and HCV genotypes among Iranian Hemophilia patients

Variables		Genotype 1	Genotype 3	Mixed genotypes	*P-value*
Age (year)		29.75±10.6	31.9±12.5	27.62±7.3	NS*
BMI		23.8±4.9	23.1± 3.7	23.81±3.2	NS*
Gender	Male	242 (70.6%)	70 (20.4%)	22 (6.4%)	NS#
	Female	16 (66.7%)	3 (12.5%)	5 (20.8%)	
HCV viral load		1527165.15± 2189149.3	1359884.92± 2728913.9	1046483.73± 1393693.2	NS*
ALT (U/L)		56.2±41	69.3±71	63.7±63	NS*
AST(U/L)		40.7±28	45.2±32	41.17±26	NS*
AlkPh (U/L)		248.9±138	226.7±135	212.9±64	NS*
AFP (ng/l)		6.07±14.7	4.6±10.6	3.9±2.7	NS**
Bleeding disorder type	Hemophilia A	181 (70.7%)	55 (21.5%)	15 (5.9%)	NS#
	Hemophilia B	34 (66.7%)	11 (21.6%)	3 (5.9%)	
	Von Willber-and	23 (67.6%)	3 (8.8%)	7 (20.6%)	
Bleeding disorder severity	Mild	18 (60%)	9 (30%)	3 (10%)	NS #
	Moderate	54 (77%)	12 (17%)	3 (4%)	
	Severe	157 (70%)	46 (20.6%)	14 (6.3%)	
Increased Spleen size		29 (80.6%)	6 (16.7%)	1 (2.8%)	NS #

1) Frequencies are shown in number (percentage) and others in Mean±SD
2) NS*: All P-Values which were calculated via Tukey HSD and Post Hoc multiple comparison Tests in one-way ANOVA analysis are non-significant (>0.05).
3) NS**: All P-Values which were calculated via Tukey HSD and Post Hoc multiple comparison Tests in one-way ANOVA analysis are non-significant (>0.05) except AFP among genotype 2 with 3 and 1 which are significant.
4) #: P-Value are calculated via Pearson Chi-Square Tests

in particular cannot be treated efficiently with IFN-α, while genotypes 2 and 3 respond favorably.[37,38] It is previously described that HCV treatment efficacy (Peginterferon plus Ribavirin) varies between 41 and 52% for genotype 1 whereas this rate was about 80% for genotype 2 and 3.[39,40] There are also some controversies about the HCV genotypes and liver disease progression but in most of them, no correlation was detected between the genotype and liver fibrosis.[41-43]

In our study, genotype 1a was the most frequent HCV genotype (58%), followed by genotype 3a (18.5%) and genotype 1b (14.7%). Mixed genotypes were also detected in 6.2% of our patients. These finding was compatible with other similar studies on HCV infected patients in Iran.[9,10,15,16,34,35,44-46] In one study among Iranian patients with inherited bleeding disorders, genotype 1a and 3a were detected in 50% and 18.2%, also mixed infection was noticed in 27.3% of patients.[3]

In USA and Western Europe, HCV genotypes 1a and 3 are more predominant, 1b and 2 in South Europe and genotype 4 in Africa and Middle East.[11,17-20] This pattern of HCV genotypes in our patients is similar to Western Europe and is different to HCV genotypes in our neighbor countries (Table 3). The reason is probably incoming clotting factors from Europe before 1987. We could not find any studies about the distribution of HCV genotype in hemophilic patients in the other countries in the Middle East (Table 4).

In present study, there was no association between HCV genotype and viral load or liver enzymes that corresponds to other studies about this association. We also showed that splenomegaly as a pending sign of portal hypertension and advance liver disease were not associated with HCV genotypes. This finding is not compatible by recent Bochud *et al.* who reported genotype 3 to be associated with accelerated liver fibrosis.[47] Sabin *et al*. previously explained that in

Table 3: HCV genotype distribution in HCV infected patients in Middle East and Europe

Author	Country	Sample size	Geno-type 1a	Geno-type 1b	Geno-type 2	Geno-type 3	Geno-type 4	Mixed	Geno-type 5	undeter mined
Abdulkarim [30]	Syria	37	46%				30%			24%
Pacsa [31]	Kuwait	144	19.5%		1.3%	6.9%	54%	4.8%		13.8%
Shobokshi [32]	Saudi Arabia	492	24.2%		7.4%	5.9%	62%	0.3%		
Ray [33]	Egypt	122	1%	1%			91%			7%
Bozdayi [34]	Turkey	36	22.2%	77.8%						
Bdour [35]	Jordan	30	40%	33.3%			26.6%			
Sharara [36]	Lebanon	142	25.3%	16.9%	4.9%	7.7%	45.7%		0.7%	
Weinstein [37]	Israel	12	16.7%	75%		8.3%				
Guadagnino [38]	Italy	148		51%	46%	3%	1%			
Harris [39]	England	567	32%	15%	8%	37%		8%		
Tuveri [40]	France	45	24.4%	26.6%	22%	22%		5%		

Table 4: HCV genotype distribution in different groups of patients in Iran

Author	Target Population	Sample size	province	Geno-type 1a	Geno-type2	Geno-type 3	Geno-type 1b	Un- de-termined genotype	Geno-type 4	Mixed
Hosseini moghad-dam [41]	Hemodialy-sis patients	66	Tehran	30.3%	-	31.3%	18.2%		16.7%	3%
Somi [42]	Hemodialy-sis patients	55	East Azarbay-jan	76.4%		5.5%	5.5%	10.9%		1.8%
Samimi-Rad [43]	Hemophilia	23	Markazi	50%	4.54%	18.2%				27.3%
Alavian [14]	thalassemia	280	Multi center	57%		35%				4%
Kabir [44]	IVDU, Transfusion history	156	Tehran	37.8%		28.9%	16.7%		1.3%	0.6%
Keyvani [45]	IVDU, Transfusion history	2231	Multi center	39.7%		27.5%	12.1%	18%		1.6%

patients with bleeding disorders, there was a correlation among HCV genotypes, viral load and serum liver enzymes.[48,49] In our patients, we failed to show this correlation in Iranian patients. Different host factors such as type of bleeding disorder, age, duration of HCV infection and ethnicity as well as different genotype distributions could underlie our different findings. It is believed that there are discrepancies in viral kinetic in different races and these discrepancies are responsible for different responses to HCV anti-viral therapy in different ethnic groups.[50]

Conclusion

Genotype 1a following by 3a and 1b are the most frequently detected HCV genotypes in Iranian hemophilic patients. This genotyping pattern is similar to western and different to Middle East countries. There was no association between HCV genotype and splenomegaly,

viral markers and liver biochemical profiles.

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Conflict of interest: None declared.

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