Original Article

Hepatitis C virus genotypes among multiply transfused hemoglobinopathy patients from Northern Iraq

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Access this article online

Website: www.ajts.org

DOI: 10.4103/0973-6247.126687

Quick Response Code:

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Abstract

Background and Aim: Owing to the scarcity of data on hepatitis C virus (HCV) genotypes in Iraq and due to their epidemiological as well as therapy implications, this study was initiated aiming at determining these genotypes in Northern Iraq. **Materials and Methods:** A total of 70 HCV antibody positive multi transfused patients with hemoglobinopathies, who had detectable HCV ribonucleic acid, were recruited for genotyping using genotype-specific nested polymerase chain reaction. **Results:** The most frequent genotype detected was genotype 4 (52.9%) followed by 3a (17.1%), 1b (12.9%) and 1a (1.4%), while mixed genotypes (4 with either 3a or 1b) were detected in 7.1%. **Conclusion:** The predominance of genotype 4 is similar to other studies from surrounding Eastern Mediterranean Arab countries and to the only earlier study from central Iraq, however the significant high proportion of 3a and scarcity of 1a, are in contrast to the latter study and may be explainable by the differing population interactions in this part of Iraq. This study complements previous studies from Eastern Mediterranean region and demonstrates relative heterogeneity of HCV genotype distribution within Iraq and should trigger further studies in other parts of the country.

Key words:

Genotype 4, genotyping, hepatitis C virus, Iraq

Introduction

Hepatitis C virus (HCV) infection constitutes an important health problem world-wide, particularly in the Eastern Mediterranean region.[1] Iraq is a large country in the latter region where previous studies reported an overall HCV prevalence reaching 3.2% among apparently healthy individuals and rates as high as 67.3% among multi transfused thalassemia patients.[2] HCV is characterized by genetic heterogeneity with at least six major genotypes, which vary in their nucleotide sequence, geographical distribution and outcome of antiviral therapy. Therefore, the determination of the HCV genotypes in a particular population is not only important as an epidemiological tool, but also as a guide to the duration and outcome of therapy and has a major impact on vaccine development.[3]

Studies on HCV genotypes among Iraqis are scarce,[2] so the current study was initiated aiming at determining the genotype distribution among multi transfused patients in Northern Iraq.

Materials and Methods

A total of 70 HCV antibody positive multi transfused patients with hemoglobinopathies, who had detectable HCV ribonucleic acid (RNA) by commercial quantitative polymerase chain reaction (PCR) kit (artus® HCV QS-RGQ — Qiagen — Germany), were referred by the Inherited Blood Diseases Center-Duhok-Northern Iraq for HCV genotyping. Patients included 46 patients with thalassemia major and 24 with sickle cell disease.

RNA was extracted from serum using QIAamp viral RNA Mini Kit (Qiagen-Germany), then HCV genotyping was performed using nested PCR amplification of the core region with genotype-specific primers designed to detect nine genotypes namely: 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a and 6a,[4] according to the manufacturer's instructions (Genekam Biotechnology AG-Germany). The serum was further used to determine the alanine and aspartate transaminase (ALT and AST, respectively) using reagents provided by Biolabo-France according to the manufacturer's instructions.

The study was approved by the ethical committee at the Faculty of Science, University of Zakho — Iraq and informed consent was obtained from all patients.

Statistical analysis utilized Chi-square and *t*-test as appropriate. $P < 0.05$ was considered to be significant.

Results

A total of 70 patients were included in the study and their age ranged between 6 and 32 years (mean

14.5 years) and male : female ratio of 1.4:1. Total number of units received till the time of sampling ranged from 10 to 290 (mean 100.8).

HCV genotyping revealed that the most common genotype detected in 37 patients (52.9%) was genotype 4 followed by 3a in 12 (17.1%), 1b in 9 (12.9%) and 1a in 1 patient (1.4%), while 5 patients (7.1%) had mixed genotypes (4 and 3a in 4.25% and 4 and 1b in 2.85%). The remaining 6 cases (8.6%) were uncharacterized by the employed genotyping procedure. [Table 1] outlines some of the main parameters in each of the genotype categories characterized. There were no significant differences in age and sex distributions between the four most frequent genotypes (4, 3a, 1b and mixed). The liver enzymes ALT and AST were highest in the genotype 3a, however, these were not significant when compared to those in genotype 4, 1b and mixed genotypes (P = 0.11, 0.65 and 0.87 respectively for ALT and P = 0.24, 0.44 and 0.47 respectively for AST). Moreover, the total number of transfusions was highest in the genotype 1b, but this was insignificant, when compared to genotypes 4, 3a and mixed genotypes (P = 0.84, 0.66 and 0.89 respectively).

Discussion

The finding that HCV genotype 4 constitutes (on its own or combined with others) 60% of the genotypes among the enrolled patients in the current study was slightly higher than the rate of 50% reported by a previous study on 48 thalassemia patients in central Iraq.[2] This finding is consistent with that reported from other Eastern Mediterranean Arab countries where genotype 4 is the most prevalent [Table 2], with Egypt having the highest prevalence world-wide.[1,5] Interestingly, a large expatriate population of Egyptians lived and worked in Iraq in the seventies and eighties of the last century and actually became part of the donor pool of the blood banks then, before the introduction of routine HCV screening and may have contributed to current genotype pattern in Iraq. The latter hypothesis could be further assessed by determining whether the genotype 4 seen among our patients is of the 4a prevalent in Egypt or it is 4c/4d subtypes prevalent in Saudi Arabia.[5] It is important to note that in contrast to Arab countries, Non-Arab countries in the Eastern Mediterranean like Iran and Turkey have a different pattern where genotype 1 predominates.[6,7]

The second genotype in frequency in the present study was 3a. This genotype is quite prevalent in Indian subcontinent where it constitutes from around a half to more than two-thirds of HCV genotypes as reported in various studies from India and Pakistan.[8,9] It is also frequently encountered in Iran, where it constitutes almost a third of the genotypes.[6] However, this genotype was not identified in any case in an earlier study on thalassemia patients from central Iraq.[2] Genotype 1a, on the other hand, was found only sporadically in the current study (1.4%), which is in contrast to an earlier study from Baghdad (27.1%) and to some reports from Jordan, Iran, Lebanon, Syria, UAE and India where it is among the most frequent genotypes, but is similar to Egypt and Saudi Arabia where it is either absent or infrequent[1,2,6,10-18] [Table 2]. Such variations in the distribution of HCV genotypes within countries is not unusual,[10,11] and is important in devising regional management strategies.

The third most common genotype found was 1b, which was also commonly encountered in an earlier study from Iraq (22.9%) . It is among the most widely distributed genotypes world-wide and comprises a major part of the HCV genotypes in the Americas,

Table 1: Some clinical and biochemical parameters in the HCV genotype categories among the enrolled patients

Parameter	HCV genotypes					
	4	3a	1b	1a	4/3a 4/1b	Uncharacterized
Number	37	12	9	1	5	6
M:F	19:18	8:4	6:3	1:0	2:3	5:1
Thalassemia/sickle	26/11	9/3	8/1	1/0	4/1	2/4
			Mean (SE)			
Age	15 (1.0)	13.8 (1.8)	14.1 (1.4)	13.0	13.6 (4.8)	14.3 (2.8)
Number of transfused units	102.2 (10.4)	98.1 (17.7)	109.9 (21)	47	92.4 (20.6)	65.7 (26.7)
S. ALT(U/L)	57.5 (7.4)	84.6 (21.7)	73.0 (18.3)	93	80.2 (8.2)	34.7 (11.6)
S. AST(U/L)	103.6 (9.0)	131.2 (34.0)	107.2 (24.3)	159	104.6 (13.0)	64.9 (15.1)

ALT: Alanine transaminase; AST: Aspartate transaminase; HCV: Hepatitis C virus; SE: Standard error

Table 2: The distribution of HCV genotypes in selected studies from Eastern Mediterranean countries and the Indian subcontinent

Country	Genotype						Reference no.
	4	3a	1b	1a	Mixed	2a	
Egypt	93	0.8	2.3	—	—	0	14
Jordan	26.6	—	33.3	40	—	—	15
India	—	43.7	14.1	11.3	—	2.8	8
Iran	1.3	28.9	16.7	37.8	1.3	—	6
Iraq (central)	35.4	—	22.9	27.1	14.6	—	2
Iraq (northern)	52.9	17.1	12.9	1.4	7.1	—	Current study
Lebanon	37	21	16	21	—	—	13
Pakistan	7.3	64.5	1.2	12.1	3.2	1.2	9
Saudi Arabia	87.5	—	3.4	2.9	—	2.4	12
Syria	54	—	27	19	—	—	16
Turkey	—	—	90	10	—	—	7
UAE	46.2	23.8	—	15	—	—	17

HCV: Hepatitis C virus

Europe, China, Australia, Japan as well as most of the surrounding Eastern Mediterranean countries [Table 2].[1-3,10]

The presence of 7.1% of mixed genotypes (genotype 4 with the two other most frequent genotypes) is not unexpected and has been observed at higher-frequency in the previous study from Baghdad and in other studies on multi transfused patients from other countries.[2,18]

The absence of significant differences in liver enzymes among various genotypes in the current study is shared by previous similar studies.[6,19] However, other studies, like that of Ijaz *et al*.[9] have documented higher ALT in genotype 4 compared to other genotypes in Pakistanis while Chakravarti *et al*.[8] documented a higher AST associated with genotype 1 among Indians. In both these studies, findings were linked to higher viral loads in the respective genotypes. One drawback in the current study is that it did not include viral load data, which may have had added some useful information.

Conclusion

It appears that about 70% of those genotyped in the current study had the less favorable genotypes (1 and 4) and thus consequently would require longer therapy durations.[3] Although the predominance of genotype 4 is similar to other studies from surrounding Eastern Mediterranean Arab countries and to earlier study from central Iraq, however, the significant high proportion of 3a and scarcity of 1a, is in contrast to the latter study and may be explainable by the differing population interactions in this part of Iraq. This study complements previous studies from Eastern Mediterranean region and demonstrates relative heterogeneity of HCV genotype distribution within Iraq and should trigger further studies in other parts of the country.

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Cite this article as: Othman AA, Eissa AA, Markous RD, Ahmed BD, Al-Allawi NA. Hepatitis C virus genotypes among multiply transfused hemoglobinopathy patients from Northern Iraq. Asian J Transfus Sci 2014;8:32-4.

Source of Support: Nil, **Conflicting Interest:** None declared.