Detection of HCV genotype 1a (HCV-1a) among patients from Al-Nasiriya city and determining its transmission risk factors

Muna R. Ahmed Warqaa Y. Salih

Abstract

Background: Hepatitis C virus (HCV) genotypes have been shown to be differently distributed between distinct geographical areas. Information's regarding hepatitis C genotypes and subtypes distribution in Al-Nasiriya city are not well known.

Objectives: The objectives of this study were to determine the frequency of various HCV genotypes in Al-Nasiriya city and the demographic and the risk factors associated with HCV transmission among Al-Nasiriya city population.

Methods: Blood samples were collected from 200 patients with chronic HCV infection who were referred from Al-Nasiriya city to the digestive disease department of Al-Yarmouk Hospital during the period from February 2019 to December 2020. Patients were screened for HCV antibodies using an enzyme-linked immunosorbent assay test. Quantitation of the HCV viral load and genotype were assessed using polymerase chain reaction (PCR).

Results: This study revealed that the most common HCV genotype was 1a (HCV-1a) with rate of 63% while (HCV-1d) showed 1.5% and (HCV-4) was 35.5% . Also the frequency of infection among females was more than males as (37.5% and 25.5%) respectively, while hemodialysis process among other transmission risk factors, increase transmission of HCV-1a infection.

Conclusions: HCV-1a is the most predominance genotype in these patients, with more prevalent in female than male over 20 years old and hemodialysis is the most risk factor in their transmission.

Keywords: (HCV-1a), Hepatocellular carcinoma, HCV genotypes, Al-Nasiriya

Introduction

Hepatitis C virus (HCV) infection constitutes a severe public health problem with approximately 80 million viraemic patients worldwide [(1)]. The infection is generally asymptomatic until late stage of liver disease is manifested. During chronic HCV infection, chronic hepatic inflammation results in progression to fibrosis and cirrhosis, which in turn predispose an individual to hepatocellular carcinoma HCC[(2)]. HCV shows a great genetic variability due to its high replication rate ,this variability has resulted, into several genotypes from (1 to 6) and subtypes denoted alphabetically a, b, c, etc. for example genotype 1a and 1b [(3)]. Genotyping plays an important role in the pathogenicity and hepatocarcinogenic effect and the therapeutic responses have been greatly influenced by these genotypes [(4)]. Such variation has great, clinical, epidemiological and economic burdens worldwide particularly among developing countries [(5)]. And the risk factors as well as intervention and preventions programs are greatly influenced by regional variation of the HCV genotypes [(6)]. Different studies have shown that HCV genotype 1 has been associated with lower rates of response to peginterferon- based regimens compared with other genotypes. Furthermore, infections with genotype 1a respond less than genotype 1b to treatment with peginterferon/ribavirin and to triple regimens that include telaprevir or boceprevir [(7-8)]. Different HCV genotypes and subtypes show distinct geographical distribution and association with risk categories in the population [(10)].

The transmission of HCV involves direct exposure to contaminated blood and is associated with intravenous drug use, iatrogenic exposures, tattooing, body piercing, and less frequently through vertical transmission and high-risk sexual behavior [(11)].This study aimed to detect the rate of HCV genotype1a in patients of Al-Nasiriya city and the risk factors involved in their transmission.

Materials and methods:

This study was conducted in Al-Yarmouk Teaching Hospital and Teaching Laboratories of Baghdad Hospital which are the main centers for receiving HCV treatment. Blood samples were collected from 200 patients with chronic HCV infection who were referred from Al-Nasiriya city to Digestive disease department of Al-Yarmouk Hospital during the period from February 2019 to December 2020. A 5 cc syringe and needle was used to collect approximate 5 mL of blood from each patient. Then, the blood samples were centrifuged at 1500 r/min for 3 min to obtain sera. The HCV antibody (HCV-AB) (fourth generation) was studied by commercial ELISA kit (DIA.PRO Diagnostic Bioprobes, Milan, Italy) following manufacturer's instruction. Then quantification of HCV RNA was done by RT-qPCR to all

patients with positive result in ELISA test. This was conducted using the Qiagen Artus HCV RG RT-PCR kit (Qiagen, Hamburg, Germany). An aliquot of 20 µL of purified sample was utilized in a total reaction volume of 50 µL. Amplification reaction for each sample and standard was performed in duplicates. Amplification cycling was performed using the Rotor-Gene Q device (Qiagen, Hamburg, Germany) [12]. Data analysis was performed with the Rotor-Gene software according to the manufacturer's instructions. We genotyped all the RT-PCR positive samples and HCV genotype and subtypes was performed by reverse hybridization technique (NLM, Milan, Italy) and primers for genotypes 1a, 1b, 1c, 3a, 3c and 4 and 2a, 2c, 3b, 5a, and 6a were incorporated [13]. A written informed consent was taken from each patient and the data sheet contained demographic information including age, gender, year of diagnosis and risk factors for HCV such as history of blood transfusion, intravenous drug abuse (IVDA), history of surgical intervention, family history of HCV positive and history of promiscuity , traditional medicine, tattoo and dental procedure.

Results:

The result of this study show high rate of HCV-1a (63%) as in figure (1) when comparing with HCV-1b that give only 1.5% and HCV-4 with rate of 35.5%. Also the frequency of infection in females more than males with (37.5% and 25.5%) respectively as in table (1) and figure (2).

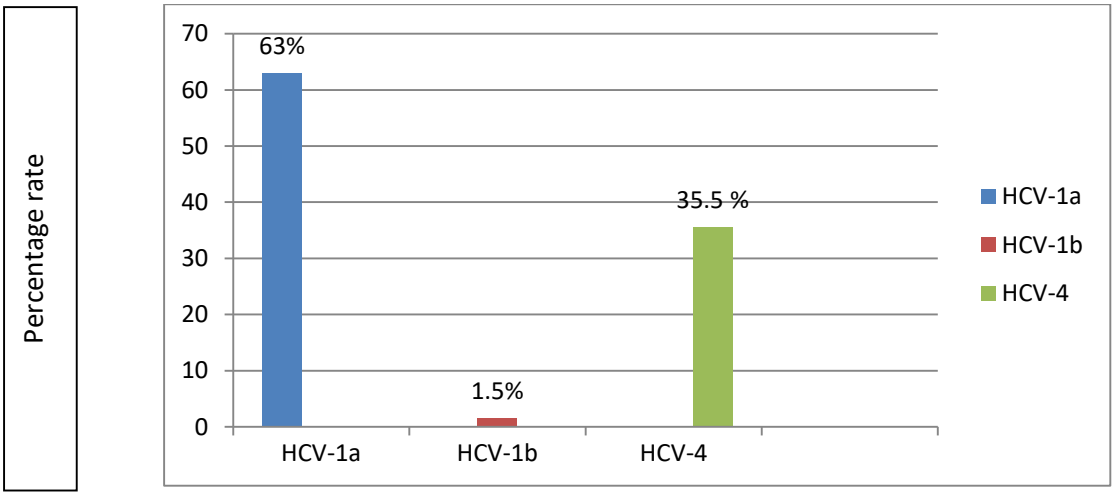
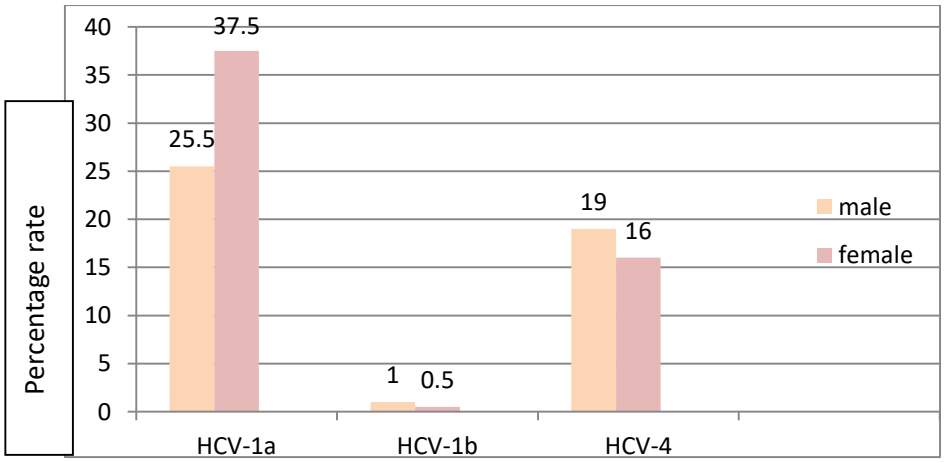


Fig.(1): The percentage rate of HCV genotype and subtype.

Table (1) The distribution of HCV-genotypes among different age and gender groups

	HCV-1a		HCV-1b		HCV-4	
age	male	female	male	female	male	female
10-19	1	2	0	0	2	3
20-29	6	21	0	1	6	5
30-39	9	19	1	0	2	1
40-49	15	11	0	0	4	7
50-59	7	13	1	0	8	12
60-69	11	8	0	0	13	5
70-79	2	1	0	0	3	0
%	25.5	37.5	1	0.5	19	16.5

Fig.(2)The influence of gender on the distribution of HCV genotypes



Genotype and subtype of HCV according to gender.

In contrast to our findings HCV prevalence decreased in individuals with age of >20 years ,while the hemodialysis process among other risk factors give slightly increasing in transmission of HCV-1a infection that described in table (2).

Table(2) Prevalence of HCV Genotype according to risk factors and gender.

	HCV-1a		HCV-1b		HCV-4		
Risk factors	male	female	male	female	male	female	count
Cesarean section	0	10	0	0	o	5	15
Intravenous drug use	1	1	0	0	2	0	4
Blood transfusion	12	6	0	0	8	4	30
Surgery	6	7	0	0	3	1	17
Dental procedure	2	2	0	0	1	0	5
Interfamilial	0	1	0	0	0	2	3
Hemodialysis	20	18	0	0	15	11	64
Un known reasons	10	30	2	1	9	10	62

Discussion:

Hepatitis C virus genotyping has been found to play an important role in understanding the epidemiology, guiding the clinical therapy, vaccine development and assessing the risk–benefit of HCV infection [14–16]. However, post transfusion transmission of HCV still remains a major health concern in multi-transfused patients [17].

In this study the finding of HCV genotype (HCV-1a) has predominant rate of 63% while HCV-4 gives 35.5% when compared this result with other study conducted in the south of Iraq, it was found that 35.4% of the samples typed as genotype 4 while 50.0% of the recruited samples typed as genotype 1 [18]. Other study among patient with hereditary blood diseases registered in Babylon, Iraq with (59% HCV-1) and (36% HCV-4) [19].

The most frequent HCV genotype reported in Arab countries, including Syria, Lebanon, Egypt, and Sudan, is genotype 4, except for Jordan and Bahrain, where genotype 1a is the most common genotype [20-22]. In contrast to our findings, HCV prevalence decreased in individuals with age of >20 years. The differences in HCV age related prevalence in different reported studies may be due to influence of the exposure to different risk factors and social behaviors in different communities and the regional differences appear to exist in the distribution of HCV genotypes [22]. Different studies in different parts of Iraq have revealed conflicting results in Basra[23] , in Diyala Province[24], and in Al-Anbar Province[25] which reported that increasing age is a risk factor for HCV seropositivity, whereas other study in Diyala did not report any such association[26].

There are several limitations of this study, including the occasional unavailability of genotype testing in the Gastroenterology Center and the cost of the test, which explains why many patients with positive PCR results do not undergo genotype testing. Another limitation is that other risk factors were not evaluated, such as other traditional medicine, tattoo, or diabetes mellitus, as disease complications and such variability are rarely studied in Iraq. So cross-sectional epidemiologic study, with stratified sampling to investigated the distribution of various HCV genotypes in Iraq and the potential links with demographic and transmission risk factors should be done.

Conclusions:

HCV-1a is the most predominance genotype in these patients in comparison with other part of Iraq with more percent in female than male over 20 years old and hemodialysis is the most risk factor in their transmission. So it was recommend continuing surveillance of blood donation centers, health-care personnel and patients, in addition to hepatitis markers screening by special type of PCR in every Digestive diseases department to diagnosis the HCV during the window period and continuous supply of anti-HCV drugs.

Reference

1. Blach S, Razavi-Shearer K, Razavi H Gower E, Este C. Global epidemiology and

genotype distribution of the hepatitis C virus infection. J Hepatol 2014; 61:S45-57.
2. Daw M, Dau A. Hepatitis C in Arab world: a state of concern. Sci World J 2012: 719494.
3. Kuiken C, Smith D. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes.. Hepatology 2013;59,issue 1/P318–327.
4. Young AM, Crosby RA, Oser CB, Leukefeld CG, Stephens DB, Havens JR Hepatitis C viremia and genotype distribution among a sample of HCV-exposed nonmedical prescription drug users in Rural Appalachia. JMed Virol 2012; 84(9):1376–1387.
5. Szabo SM, Bibby M, Yuan Y, Donato BM, Jiménez-Mendez R, Castaeda- Hernndez G *et al*. The epidemiologic burden of hepatitis C virus infection in Latin America. Ann Hepatol 2012; 11(5):623–635.
6. Westbrook RH, Dusheiko G. Natural history of hepatitis C. J Hepatol 2014;61: 558---68.
7. Jacobson IM, McHutchison JG, Dusheiko G, Di Bisceglie AM, Reddy KR,Bzowej NH *et al*. Telaprevir for previously untreated chronic hepatitisC virus infection. N Engl J Med 2011 ; 364 (25):2405–24016.
8. Poordad F, McCone J Jr, Bacon BR, Bruno S, Manns MP, Sulkowski MS *et al*. Boceprevir for untreated chronic HCV genotype 1 infection. N Engl J Med 2011; 364(13):1195–1206.
9. Fried MW, Shiffman ML, Reddy K, *et al*. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002; 347(13):975–982.
10. Messina JP, Humphreys I, Flaxman A, *et al*. Global distribution and prevalence of hepatitis C virus genotypes. Hepatology 2014; 61(1):77–87.
11. Dunford L, Carr MJ, Dean J, Waters A, Nguyen LT, Ta Thi TH, *et al.* Hepatitis C virus in vietnam: High prevalence of infection in dialysis and multi-transfused patients involving diverse and novel virus variants. PLoS One 2012; 7(8):e41266.
12. Idrees M, Hussain A, The first complete genome sequence of HCV-1a analysis with complete genome. Virol J 2018; 10:211.
13. Zein NN. Clinical significance of hepatitis C virus genotypes and subtypes. Clin.Microbiol Rev 2000; 13:223–235.
14. Halliday J, Klenerman P, Barnes E .Vaccination for hepatitis C virus closing in on an evasive target. Expert Rev Vaccines 2018; 10:659–672.
15. Pybus OG, Barnes E, Taggart R, Lemey P, Markov PV, Rasachak B *et al.* Genetic history of hepatitis C virus in East Asia. J Virol 2016;83:1071–1082.
16. Simmonds P, Bukh J, Combet C, Deléage G, Enomoto N, Feinstone S *et al*. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology 2018; 62:362–371.
17. Krawczynski K, Judson FN, Mares A, Alexander WJ, Alter MJ, Margolis HS *et al*. The natural history of community-acquired hepatitis C in the United States. The sentinel countis non-A non-B hepatitis study team. N Engl J Med 2016; 273: 9019-589.
18. Al-Kubaisy W, Al-Naib KT, Habib M. Seroprevalence of hepatitis C virus specific antibodies among Iraqi children with thalassaemia. East Mediterr Health J 2006; 12(1–2): 204-210.
19. Alawadi NB. Prevalence of hepatitis B and C virus infections among Iraqi patients registered to Babylon center for inherited blood disorders. Int J Adv Res 2014; 2:383-388.
20. Ramia S, Eid-Fares J. Distribution of hepatitis C virus genotypes in the middle east. Int J Infect Dis 2016;12:162-167.
21. Janahi EM, Al-Mannai M, Singh H, Jahromi MM. Distribution of hepatitis C virus genotypes in Bahrain. Hepat Mon 2015; 15:e30300.
22. Abdulghani M A, Gailan A, Amina H A. Seroprevalence of Hepatitis C Virus in Iraqi Population. JOJ Immuno Virology 2016;1(3):555565.DOI: 19080/JOJIV.2016.01.555565 **008**.
23. Oula Abdullah Najim, Meaad Kadhum Hassan, Najim OA, Hassan MK. Prevalence of hepatitis C virus seropositivity among multitransfused patients with hereditary anemias in Basra, Iraq. Iraqi J Hematol 2018; 7: 39-44.
24. Raham TF, Wahed SS, Alhaddad HN. Prevalence of hepatitis C in Diyala-Iraq. Iraqi Acad Sci J 2017; 31:43-62.
25. Al-Doori AM. Prevalence of hepatitis B and hepatitis C among blood donors in Al Anbar governorate. Fac Med Baghdad 2016; 48:149-151.
26. Noaman NG. Prevalence of hepatitis C virus infection among blood donor and certain risky groups in Diyala province. Diyala J Med 2018; 4:22-33.