DOI Number: 10.37506/v20/i1/2020/mlu/194454

The Distribution of Hepatitis C Virus Genotypes, Viral Load and Antibody Titer among Iraqi Chronic Hepatitis Patients

Abdel Wahab A. R. Alshaikhly[1], Zaidoon A. Musa[2], Ban J. Qasim[3], Haider F. Ghazi[4], Wisam J. Mohammed[1]

[1]Department of Pharmacy, Al Rasheed University College, Baghdad, Iraq, [2]Consultant Pathologist Physician, Al-Emamain Al-Kadhimain Medical City, [3]Departments of (Pathology and Forensic Medicine, [4]Microbiology, College of Medicine, Al-Nahrain University.

Abstract

Background: The infection by hepatitis C virus causedliver diseases such as:chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. The current study aims to investigate the rate of HCV genotypes, subtypes, viral load and antibody immune response among Iraqi chronic hepatitis patients.

Methodology: During 2012-2017 period seventy-hundred and twenty tow hepatitis patients were referred to Middle East Laboratory from Baghdad and other provinces asking for HCV viral load, its genotype and anti-HCV antibody determination.

Results: A cross six years records, HCV genotype 4 was the most prevalent among Iraqi chronic hepatitis patients (46.68%), then genotype 1 (37.12%).There is notable changes in HCV genotypes distribution especially during 2017, where the genotype 1 predominantly found (52.63%). Although, these genotypes were none significantly associated with gender, age, viral load or anti-HCV antibody.

Conclusion: This recordindicates a recent change in the rate of HCV genotype 1 over genotype 4 infection in Iraqi chronic hepatitis patients.

Key words: *HCV, genotypes, Chronic hepatitis.*

Introduction

The hepatitisCvirus (HCV) one of the Flaviviridae members belonging to the genus Hepacivirus (45,34). Nearly, 140 million subjectswere considered as chronic HCV infectedpateints 36. HCV can consequently causes acute and/or chronic hepatitis, liver cirrhosis or hepatocellular carcinoma 26. The virus classified into seven genotypes (numbered 1 to 7), with multiple subtypes (e.g., subtype 1a, 1b, and so on) 51. The distribution of HCV genotypes and subtypes have been reported across the world. Genotypes 1 and 2 were reported the mostcommoninNorth America, Japan, and Europe, whereas genotype 3 is mostly found in Southeast-Asia and India. Genotype 4 was the most common genotype in Middle Eastern countries, Egypt, Syria, and Saudi Arabia. Genotype 5 wasmostcommonlyreported in South Africa(26, 35 ,22). HCV vaccination is so difficult because of highly genetic diversity of HCVgenotypes (20,24). In other hand, determination of an HCV genotype in patents is important for the management and treatment 49. Moreover, identification of HCV genotypes in chronic hepatitis C patientsis importantfor more aggressive therapeutic management of certain patients. Both genotype 2 and 3 were reported to be more responsive to pegylated-interferon (PEG-IFN) and ribavirin combination therapy than those patients with genotype 1 and 4, thus require different treatment duration and dose(46 ,41, 19).

In the past twenty years studies showed that viral load can be considered an indicator of response to antiviral therapy and higher viral load could be related to lower rate of response to therapy (37,14,21,17, 44,47).

This study was performed6 years analysis of HCV viral load, genotypes, subtypes and anti-HCV antibody titerin Iraqi chronic hepatitis patients.

Materials and Method

Sample collection and setting:

A total of seventy hundred and twenty-two blood samples were collected fromchronic hepatitispatientsconsulted by a specialist doctors referred to Middle East laboratory from Baghdad and other governorates during five years (2012-2017). Those patients were taken of all ages and both gender types, different residences and occupations.

Patients age, gender, possible cause of infection, HCV viral load, genotypes and anti-HCV antibody were determined for all patients.

Extraction of viral Nucleic acid:

After separation of serum sample, Viral RNA was extracted from each patient using specialized kit (Roche/ Germany) Cat. No. 11 858 874 001.

Determination of HCV viral load:

COBAS-TaqMan- HCV testing method used to determine the HCV RNA load (Roche, German). The working master mix solution that used in the real time PCR was prepared as follows:

1- For 12 tests, 669 µl of HCV master mixwere removed and placed in a 2 ml tube.

2- Of CTM Mn^{+2}, 81 µL were added to the 2 ml tube containing HCV master mix, the tube was cap and mixed well by inverting 10 times, the working master mixwas protected from light and used within 1 hour.

3- Of working master mix, 50 µl were pipette into each K-tube or K-tray well.

4- From each serum sample, 50 µl added to K-tube or K-tray wells that contain working master mix.

5- Each specimen and control was gently mixed up and down three times with the micropipettor without generating bubbles.

6- The step 3 was repeated for each processed specimen and processed control until all have been transferred to K-tube and operated on COBAS Tagman 48 analyzer.

Determination of HCV genotype in chronic infected patients with HCV:

After optimizations, the dispense 6µl of master mix was mixed gently in the previously marked 0.2 ml test tubes and 14µl of extracted RNA were added to each tube and mixed carefully. The following program was run as: reverse transcription for 15 minutes at 50°C, initial denaturation at 95°C for 20 seconds, then fifty cycles of denaturation at 95°C for 15 seconds and annealing at 60°C for 60 seconds, the final step stop at 4°C.

After hybridization, the color development achieved before proceeding the interpretation of results.

Determination of anti-HCV antibody titer:

The anti-HCV was determined using electrochemiluminescence immunoassay "ECLIA" cobas e 411 analyzer. This *in vitro* diagnostic test routinely used for determination of Antti-HCV IgG antibody in serum or plasma samples.

Statistical analysis

The statistical analysis done by using GraphPad Prism7® software. Mean and standard deviation in addition to median and 25-75 confidence interval for numerical data. Frequency and percentage were calculated for categorical data. Chi-square used to estimate the possible association between studied parameters.

Results

The mean agewas 37.49±13.82 years old. The male patients were 337/722 (46.68) while female was385 (53.32). As a possible cause, 83 patients have a history of blood transfusion (11.5), followed by 58 patients whom undergone a surgery (8.03) and visiting dentist constitutes 48 patients (5.3%). The mean anti-HVC IgG is 53.01±37.16 and the mean viral load is $5\text{^}10^6 \pm 3\text{^}10^7$.

In a total of 5 years genotype 4 was most frequent genotype comprising of (46.68), genotype 1 (37.12), genotype 3 was %, genotype 2 was 3.32 % and only 1 case (0.14%) was genotype 5.

Table 1: Patients characteristics and serum anti-HCV antibody, viral load and genotypes.

		Value
Age years (mean±SD)		37.49±13.82
Median (25-75 percentile)		43.74 (22-63)
Sex (%)	Male	337 (46.68)
	Female	385 (53.32)
Possible causes (%)	Blood	83 (11.5)
	Dentist	48 (6.65)
	Surgery	58 (8.03)
	Unknown	533 (73.82)
HCV Ab (mean±SD)		53.01±37.16
Median (25-75 percentile)		42.75 (30.6-60.7)
HCV viral load (copy/ml) (mean±SD)		5.E+06±3.E+07
Median (25-75 percentile)		2.E+05 (7.E+03-2.E+06)
Genotype (%)	1	268 (37.12)
	2	12 (1.66)
	3	24 (3.32)
	4	337 (46.68)
	5	1 (0.14)
	Not determined	80 (11.08)
Subtype (%)	1	151 (20.91)
	1a	86 (11.91)
	1b	31 (4.29)
	2	11 (1.52)
	2b	1 (0.14)
	3	15 (2.08)
	3a	8 (1.11)
	3b	1 (0.14)
	4	289 (40.03)
	4a	41 (5.68)
	4h	7 (0.97)
	5	1 (0.14)
	Not determined	80 (11.08)
Total		722 (100%)

Table 2: Association between HCV genotypes with years of analysis, Sex, median viral load and median antibody titer.

1		HCV genotype						P value
		2	3	4	5	Unknown		
2012 (n=140)		36	8	4	77	1	14	<0.001**
%		25.71%	5.71%	2.86%	55.00%	0.71%	10.00%	
2013 (n=201)		73	3	10	97	0	18	
%		36.32%	1.49%	4.98%	48.26%	0.00%	8.96%	
2014 (n=94)		44	0	2	35	0	13	
%		46.81%	0.00%	2.13%	37.23%	0.00%	13.83%	
2015 (n=137)		54	1	3	70	0	9	
%		39.42%	0.73%	2.19%	51.09%	0.00%	6.57%	
2016 (n=93)		31	0	3	43	0	16	
%		33.33%	0.00%	3.23%	46.24%	0.00%	17.20%	
2017 (n=57)		30	0	2	15	0	10	
%		52.63%	0.00%	3.51%	26.32%	0.00%	17.54%	
Sex type	Female (n=385)	137	4	9	194	1	40	0.129NS
	%	35.58%	1.04%	2.34%	50.39%	0.26%	10.39%	
	Male (n=337)	131	8	15	143	0	40	
	%	38.87%	2.37%	4.45%	42.43%	0.00%	11.87%	
HCV viral load	Median	7.00E+05	2.00E+05	3.00E+05	2.00E+05	2.00E+06	9.00E+05	0.096NS
	Percentile 25	7.00E+04	1.00E+04	6.00E+04	2.00E+04	2.00E+06	5.00E+05	
	Percentile 75	4.00E+06	3.00E+05	8.00E+05	2.00E+06	2.00E+06	6.00E+06	
HCV Ab	Median	38	29	41.75	51	46.33	48.9	0.435NS
	Percentile 25	27	24	26.6	34.9	46.33	32.5	
	Percentile 75	51	64	53.5	64.3	46.33	58.4	

NS=None statistical significant difference ($p > 0.05$).

**= High statistical significant difference ($p \leq 0.001$).

Discussion

HCV infection sill a major health problem in Iraq during the five years of analysis. Several records on HCV genotypes suggesting genotype 1 were predominantly distributedworldwide 42, some of these are limited to certain geographical areas 50.It's still that no available data have described the HCV genotypes among Iraqis patients. Otherwise,many of these records lack the validity due to non-representative sample size or dealing with responsiveness to treatment.

Also, AL-Mula, et al., 2013 collected asymptomatic patients from hospitals in Najaf, Babylon, Qadisyia, Karbala and Baghdad governorates and reports the predominance of genotype 4 9 .

Al-Kubaisy, published 4 articles related to age of pregnancy 5, coinfection with HIV among hemophilia patients 7, Hepatocellular carcinoma 6, as well as history with miscarriage among pregnant females 8.

HCV genotypes distribution in the Arab gulf region reported that genotype 4 was the most common in most countries in this region, followed by genotypes 1 and 3 like, Kuwait 34 . Saudi Arabia (53,13), Egypt and is common in other MENA countries such asJordan, Lebanon, and Syria(48, 23, 15).

In contrast to HCV genotype distribution in Iraq and other middle east countries, whereas genotype 4 the predominant one. Genotypes 1 and 3 are the most common genotypes in India, Nepal, and Pakistan, and there are large expatriate populations from these countries in the Arabian Gulf region 39 like Bahrain 28 and Dubai 2, Oman 4 it was reported that the higher rate was genotype 1 and 3. Also, different Asian populations have been reported to be infected with HCV genotype3 like: Thailand, Malaysia, India and Pakistan 25, Mainland China(29,16).In our data, only one case have been classified to be caused by genotype 5a which is rarely reported in Punjab 3, Syria 11 , Genotypes 5 and 6 were confined to South Africa and Southeast Asia.

Of note, our data reported a highly statistically significant change of increasing distribution of genotype 1 over genotype 4 (table 2). This might be due to changing the source of infection from areas or population had have different genotype or high rate of immigrants during later years(18).

Our records didn't find an association between HCV genotype and sex distribution, this comes in agreement with studies like Alfaresi, 2011 10, Abdel-Moneim, et al., 2012 1 and

This study was the largest study in Iraq that find out that genotype 4 as a predominant genotype over last years with significant change to genotype 1 during 2017 among Iraqis chronic hepatitis patients.

Conclusion

This record indicates a recent change in the rate of HCV genotype 1 over genotype 4 infection in Iraqi chronic hepatitis patients.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Pharmacy, Al Rasheed University College, Baghdad, Iraq and all experiments were carried out in accordance with approved guidelines.

References

1. Abdel-Moneim A S. HCV infection among saudi population: High prevalence of genotype 4 and increased viral clearance rate', PLoS ONE, 2012;7(1),:5–10.
2. Abro AH, Al-Dabal L, Younis NJ. 'Distribution of hepatitis C virus genotypes in Dubai, United Arab Emirates.', JPMA. The Journal of the Pakistan Medical Association, 2010;60(12): 987–90.
3. Afridi S. Molecular epidemiology and viral load of HCV in different regions of Punjab, Pakistan', Virology Journal, 2014;11(1): 2–6.
4. Al-Busafi SA. Epidemiology of chronic hepatitis C infections at a tertiary care centre in Oman', Sultan Qaboos University Medical Journal, 2017 ;(4):e404–e410.
5. Al-Kubaisy W. Specific HCV antibodies, RNA, and genotypes detection correlated to the age of pregnant women in Iraq', International journal of Infectious Diseases, 2012;16:201317–473.
6. Al-Kubaisy W. Hepatitis C Virus Prevalence and Genotyping among Hepatocellular Carcinoma Patients in Baghdad', Asian Pacific Journal of Cancer Prevention J Cancer Prev, 2014;15(1518):7725–7730.
7. Al-Kubaisy WA , Al-Naib K T , Habib MA. 'Prevalence of HCV/HIV co-infection among haemophilia patients in Baghdad', Eastern Mediterranean Health Journal, 2006;12(3–4): 264–269.
8. Al-Kubaisy WA , Niazi AD, Kubba K. 'History of miscarriage as a risk factor for hepatitis C virus infection in pregnant Iraqi women', Eastern Mediterranean Health Journal, 2002;8(2–3): 239–244.
9. AL-mola G, Tarish H, Abdulsada K LR.

'Investigation of the significant HCV genotype in asymptomatic patients in Iraq with the use of Reverse-Transcription PCR.', Int. J. Sci. Engg. Res., 2013;4(12):1306–13.

10. Alfaresi MS. 'Prevalence of hepatitis C virus (HCV) genotypes among positive UAE patients', Molecular Biology Reports, 2011;38(4): 2719–2722.
11. Antaki N. The unexpected discovery of a focus of hepatitis C virus genotype 5 in a Syrian province', Epidemiol. Infect., 2009;137:79–84.
12. Bakr I. Higher clearance of hepatitis C virus infection in females compared with males', Gut, 2006;55(8):1183–1187. doi: 10.1136/gut.2005.078147.
13. Bawazir A, et al. 'Hepatitis C virus genotypes in Saudi Arabia: A future prediction and laboratory profile', Virology Journal. Virology Journal, 2017;14(1):1–8. doi: 10.1186/s12985-017-0873-7.
14. Bell H. Genotype, viral load and age as independent predictors of treatment outcome of interferon-alpha 2a treatment in patients with chronic hepatitis C. Construct group', Scand.J Infect.Dis., 29(0036-5548 SB-M), 1997;17–22.
15. Chemaitelly H , Chaabna K, Abu-Raddad LJ . 'The epidemiology of hepatitis C virus in the fertile crescent: Systematic review and meta-analysis', PLoS ONE, 2015;10(8).
16. Chen Y. Hepatitis C virus genotypes and subtypes circulating in Mainland China', Nature Publishing Group. Nature Publishing Group, (July), 2017;1–7.
17. Chevaliez S , Pawlotsky JM. Interferon-based therapy of hepatitis C', Advanced Drug Delivery Reviews. Elsevier, 2007;1222–1241.
18. Chlabicz S. Changing HCV genotypes distribution in Poland-Relation to source and time of infection', Journal of Clinical Virology, 2008;42(2): 156–159.
19. Coppola N. Efficacy of pegylated interferon α-2a and α-2b in patients with genotype 1 chronic hepatitis C: A meta-analysis', BMC Infectious Diseases. BMC Infectious Diseases, 2012;12(1):1. doi: 10.1186/1471-2334-12-357.
20. Dahari H , Feinstone SM, Major ME. Meta-analysis of hepatitis C virus vaccine efficacy in chimpanzees indicates an importance for structural proteins', Gastroenterology. W.B. Saunders, 2010;139(3): 965–974.
21. Fanning L. Viral load and clinicopathological features of chronic hepatitis C (1b) in a homogeneous patient population.', Hepatology (Baltimore, Md.), 1999;29(3):904–7.
22. Ghaderi-zefrehi H. The Distribution of Hepatitis C Virus Genotypes in Middle Eastern Countries : A Systematic Review and Meta-Analysis', 2016;16(9).
23. Hamoudi W , Al-Smadi S, Lutfi K. Durability of Sustained Virological Response and Long Term Follow Up to Pegylated Interferon and Ribavirin in Treated Patients with Chronic Hepatitis C', Journal of the Royal Medical Services, 2014;21(2):6–12.
24. Hesamizadeh K. Next Steps Toward Eradication of Hepatitis C in the Era of Direct Acting Antivirals', Hepatitis Monthly, 2016;16(4). doi: 10.5812/hepatmon.37089.
25. Ho SH. Genotype 3 is the predominant hepatitis C genotype in a multi-ethnic Asian population in Malaysia', Hepatobiliary and Pancreatic Diseases International. The Editorial Board of Hepatobiliary & Pancreatic Diseases International, 2015;14(3): 281–286. doi: 10.1016/S1499-3872(15)60363-0.
26. Hoofnagle JH. 'Course and outcome of hepatitis C', in Hepatology. Wiley-Blackwell, 2002; s21–s29. doi: 10.1053/jhep.2002.36227.
27. Hussein NR. The distribution of HCV in subjects attending hospitals in Duhok City, Iraq', Asian Pacific Journal of Tropical Biomedicine. Elsevier B.V., 2017;7(3):pp. 262–264. doi: 10.1016/j.apjtb.2016.12.003.
28. Janahi EM. Distribution of hepatitis C virus genotypes in Bahrain', Hepatitis monthly, 2015;15:1–7. doi: 10.3109/00365549709008660.
29. Ju W. Hepatitis C virus genotype and subtype distribution in Chinese chronic hepatitis C patients: Nationwide spread of HCV genotypes 3 and 6 Hepatitis viruses', Virology Journal. Virology Journal, 2015;12(1):1–6. doi: 10.1186/s12985-015-0341-1.
30. Kadhem Manuti J , Sh AL-Obeidy E, Abdul-Rasheed O F. Distribution of Hepatitis C Virus (HCV) Genotype among Iraqi Hemodialysis Patients', International Journal of Advanced Research Journalwww.journalijar.com INTERNATIONAL JOURNAL OF ADVANCED RESEARCH, 2014; 2(1): 442–445. Available at: http://www.journalijar.com/uploads/651_IJAR-

2491.pdf (Accessed: 5 November 2017).

31. Kareem BO, Salih GF. 'HEPATITIS C VIRUS GENOTYPING IN SULAIMANI GOVERNORATE', European Scientific Journal, 2014;10(15):1857–7881. Available at: http://eujournal.org/index.php/esj/article/viewFile/3466/3229 (Accessed: 1 December 2017).
32. Khalid MD, Abdullah BA. 'HEPATITIS C VIRUS GENOTYPES IN IRAQ', 2012; 11(2):475–480. Available at: https://www.iasj.net/iasj?func=fulltext&aId=71879 (Accessed: 1 December 2017).
33. Khdeir NA, et al. 'Genotyping of Hepatitis C Virus (HCV) in Patients of Basra Province/Iraq', International Journal of Innovative Research in Science, Engineering and Technology (An ISO Certified Organization), 2007;3297(6). doi: 10.15680/IJIRSET.2015.0506156.
34. Kuiken C, Simmonds P. Nomenclature and Numbering of the Hepatitis C Virus'. Humana Press, 2009;33–53. doi: 10.1007/978-1-59745-394-3_4.
35. Lee C-M , et al. 'Hepatitis C virus genotypes: clinical relevance and therapeutic implications.', Chang Gung medical journal, 2007;31(1):16–25. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18419050.
36. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans VAJ. 'WHO | Hepatitis C', WHO. World Health Organization. Available at: http://www.who.int/mediacentre/factsheets/fs164_apr2014/en/ (Accessed: 29 July 2018). 2015.
37. Martinot-Peignoux M. Pretreatment serum hepatitis C virus RNA levels and hepatitis C virus genotype are the main and independent prognostic factors of sustained response to interferon alfa therapy in chronic hepatitis C', Hepatology, 1995;22(4 Pt 1):1050–1056.
38. Messina JP. Global distribution and prevalence of hepatitis C virus genotypes', Hepatology, 2015;61(1):77–87.
39. Mohamoud YA , Riome S , Abu-Raddad L J. Epidemiology of hepatitis C virus in the Arabian Gulf countries: Systematic review and meta-analysis of prevalence', International Journal of Infectious Diseases. International Society for Infectious Diseases, 2016;46: 116–125.
40. Mujahid A, Ph A. (no date) 'Genotyping of Hepatitis C Virus Isolates from Iraqi Hemodialysis Patients by Reverse Transcription-PCR and One Step Nested RT-PCR 2014;3(1).