Prevalence of HCV Genotypes in Correlation with Viral Load in Northern Region of Iraq

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ABSTRACT

Background: Hepatitis C virus (HCV) is a globally prevalent pathogen with a diverse genotype distribution. The purpose of this study was to determine the prevalence of HCV genotypes and their relationships to sociodemographic traits and viral loads in patients from Iraq's northwestern areas.

Methods: 212 patients from six governorates in Iraq who had been sent to the Genome Diagnosis Laboratory after receiving positive findings for anti-HCV antibody and HCV-RNA tests participated in a cross-sectional study (GDL). Only samples with a positive result for HCV- RNA were selected for detection of HCV genotypes and viral load.

Results: Most of the cases were from Erbil, Kirkuk and Sulaimania, with the highest prevalence being genotype 1a (29.2%) followed by genotypes 4 and 1b (23.6% and 22.7%, respectively). Genotype distribution was not significantly different among various age groups (P= 0.067). The overall high viral load was observed in younger age groups particularly in males. The overall distribution of viral load among genotypes in relation to patient age and gender did not, however, show any statistically significant variations (P= 0.86 and 0.3, respectively).

Conclusion: The genotypes 1a, 4 and 1b were more prevalent among the HCV patients. High viral load was observed in younger age groups particularly in males.

Keywords: Hepatitis C infection, Genotyping, Viral load

INTRODUCTION

HCV is a pathogen that is common around the world and is the number one cause of mortality and morbidity. According to the most recent estimates of disease burden, there are more than 185 million infections worldwide and a rise in sero-prevalence of 2.8% during the previous 15 years[1,2]. Chronic HCV patients usually develop liver cirrhosis, which increases their chance of getting hepatocellular carcinoma. Worldwide, the prevalence of HCV infection ranges from 0.2% to 40% (between 0.2% and 2.2% in wealthy nations and close to 7% in underdeveloped nations). Since Egypt has recorded the greatest frequency of the disease (18.1%), the Middle East is recognized to have a particularly high rate of HCV infection[3]. The HCV prevalence in Iraq has been reported with a range of 0.32% to 7.1% in various studies[4]. HCV shows high genetic heterogeneity with six distinct genotypes throughout the world each containing multiple subtypes. Genotypes 1, 2, and 3 are widespread throughout the world, but genotypes 4, 5, and 6 are more regionally restricted[5,6]. Genotype 4 is common to the Middle Eastern countries while subtype 5a is mainly found in the Southern part of Africa and genotype 6 preponderates in China and Southeast Asia[7]. Genotyping can be used to predict treatment response and determine treatment duration[8-10]. The prognostic and treatment planning benefits of HCV genotyping are real[11,12]. According to research[13,14], patients with genotype 1 chronic HCV are more likely to experience the advancement of liver disorders. Monitoring treatment responses and relapse rates can both benefit from knowing virus loads. Since viral counts at the time of diagnosis are associated with treatment requirements and durations, both viral load and HCV genotype may have clinical relevance in persistently infected persons[2]. The main causes of the apparent inexorable growth in HCV infection in many developing countries, however, have thus far been lack of knowledge, insufficient blood screening facilities, nosocomial transmission, and a lack of effective treatments (due to a variety of reasons)[15]. Due to a dearth of medical facilities in impoverished nations, the diagnosis, prognosis, interferon therapy, and clinical care of HCV are all very poor. The current work has been conducted to document the prevalence of HCV genotypes and their association with socio-demographic characteristics and viral load in patients from different governorates of Iraq. Epidemiological data are the foundation for the development of preventive strategies able to eradicate HCV infection. Because of this, improved understanding of the HCV genotype distribution in this area can encourage better treatment plans and possibly enhance the care of patients with HCV infection.

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PATIENTS AND METHODS

Between April 2015 and March 2018, 212 individuals were involved in this cross-sectional study who had received referrals to the Genome Diagnosis Lab and the Erbil Public Health Lab with positive anti-HCV antibody and HCV-RNA test results.

SAMPLE COLLECTION

Each patient gave their informed consent after being educated about the study. HCV- infected patients who attended central public health laboratories in six governorates of Iraq (Erbil, Duhok, Suleymania, Anbar, Neinawa, and Kirkuk), who referred to GDL were recruited. Their age, sex, address, marital status, and other demographic details, as well as any problems, were recorded. Positive results for plasma genomic HCV- RNA and anti-HCV antibodies served as inclusion criterion. Demographic information and HCV infection risk factors, such as transfusion, tattooing, criminal history, a positive family history, IV drug use, and hazardous sexual conduct, were documented for each patient in the study. Approximately, 5mL venous blood was taken and mixed at ratio of 20:1 with 3% EDTA solution. Plasma was extracted within 6 h of blood collection by centrifugation. The collected plasma was instantly used for HCV-RNA extraction and stored at -80ºC for future use. The handling of biological specimen was done according to the Declaration of Helsinki criteria[16].

HCV-RNA EXTRACTION AND QUANTIFICATION

RealLine HCV Quantitative – Uni-Format (BIORON Diagnostics, Germany) was used for RNA extraction and quantification. Briefly, 100 µL of plasma was added to 30 µL of Internal Control (IC). Positive and negative controls were made accordingly. 500 µL of Lysis reagent was added and mixed vigorously then incubated at 56ºC and 1300 rpm for 10 min into Thermo Shaker followed by addition of 600 µL of nucleic acid precipitation solution. The suspension was centrifuged at 13000 rpm for 5 min after being incubated at room temperature for 5 min, and the supernatant was then discarded. The pellet was centrifuged and mixed twice with 500 L of washing solution. The pellet was dried at RT for 2 min then dissolved in 200 µL of nuclease free water via incubation at 56ºC and 1300 rpm for 10 min. 50 µL of extracted RNA was mixed with Ready Master Mix (RMM) and the rest was stored at -20ºC for genotyping. Triplet PCR-tubes for each sample were made by adding 20 µL of reaction. Sample, controls and standard tubes were placed into Rotor-Gene 6000 (Corbett Research, Australia) for reverse transcription (45ºC for 30 min) and real-time PCR (94ºC for 1 min Polymerase activation, 94ºC for 1min and 60ºC for 10 sec repeated 50 cycles). Fluorescence measurement was done at 60ºC. Collection of real-time PCR amplification data for IC was done through FAM channel and for HCV-cDNA through ROX channel. The World Health Organization for Hepatitis C Virus (NIBSC Code: 96/798) measured the quantity of HCV RNA in relation to 10fold serial dilutions of Positive Control in each run and expressed the results in international units (IU/ml).

HCV GENOTYPING

Only samples with positive result for HCV-RNA were selected for detection of HCV genotypes 1a, 1b, 2, 3a, 4, 5a and 6 using HCV-genoytpe-FRT (AmpliSens, Russia). It is a two-step PCR for reverse transcription and real-time PCR.

Reverse Transcription: Master Mix was prepared by adding 6 µL of reverse transcriptase (MMLv) to reaction mixture (RT-mix) then 10 µL was mixed with 10 µL of RNA sample. The reaction was incubated at 37ºC for 30 min to produce cDNA.

Real-time PCR: Each cDNA sample was analysed with the use of 4 reaction mixtures of 1b/3a, 1a/2, IC/4, and 5a/6. The reaction was made by mixing 10 µL of cDNA 15 µL of prepared mixture. Reactions were placed in Rotor-gene 6000 with amplification program as 95ºC for 15 min (hold), 95ºC for 5 sec, 60ºC for 20 sec, and 72ºC for 15 sec (5 cycles), 95ºC for 5 sec, 60ºC for 20 sec, and 72ºC for 15 sec (40 cycles). The detection of HCV genotypes for each of the reactions mixtures was done via setting FAM channel (Green) for 1b, 1a, 1c, and 5a genotypes, and HEX channel (Yellow) for 3a, 2, 4, and 6 genotypes.

DATA ANALYSIS

Data entry, analysis and graphing were done by using SPSS v20, GraphPad Prism v6, and R Studio v3.5.1 packages. Two approaches were used; descriptive statistics for determining the frequencies and percentages, additionally, we employ the Chi square association test for categorical variables in the second method. P value less than 0.05 is considered statistically significant.

RESULTS

The study had 212 patients in all. The sample's mean age SD was 33.9 11.8 years, ranging from 12 to 62 years. About 57 % of them was males with a male to female ratio of 1.3:1. Most of the cases were from Erbil, Dahuk and Sulaimania, (34.9%, 18.4% and 17.0% respectively).

Genotype Distribution According to Socio-Demographic Characteristics of the Patients: The study revealed that genotype 1a was more prevalent among those aged 30-39 years and those aged 40-49 years (29.0% and 22.6%, respectively), genotype 1b and genotype 3 was more prevalent among those aged 20-29 years (31.9% and 28.2%, respectively), while genotype 4 was more prevalent (36.0%) among those aged 30-39 years. However, mixed genotype infection was reported among only 10% of the patients with a statistically non-significant association (P= 0.067). In contrast, the genotype distribution was substantially associated with the genotype distribution (P = 0.001) but not with the gender of the patient (P=0.445), showed a diverse pattern across the residency of the patients, it was more prevalent in Erbil, followed by Sulaimania and Duhuk (34.9%, 18.4% and 17.0%, respectively), (Table 1 and figure 1).

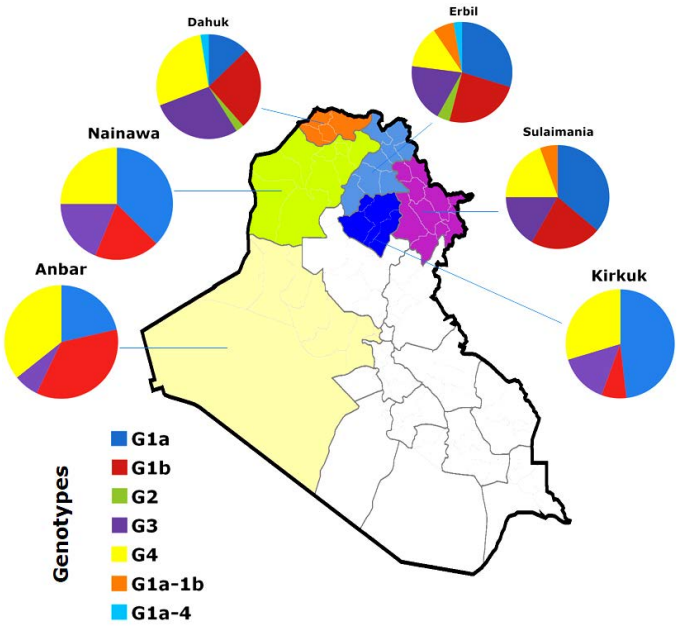

Figure 1: HCV genotypes distribution across North Western governorates of Iraq

Table 1: Genotype distribution according to the socio-demographic characteristics of the patients

Variables	Genotypes							P value **
	Gt.1a No. (%)	Gt.1b No. (%)	Gt. 2 No. (%)	Gt. 3 No. (%)	Gt. 4 No. (%)	Mixed No. (%)	Total * No. (%)	
Age groups in years								
< 20	6 (9.7)	4 (8.5)	0 (0.0)	6 (15.4)	5 (10.0)	5 (50.0)	26 (12.3)	0.067
20 – 29	13 (21.0)	15 (31.9)	1 (25.0)	11(28.2)	16 (32.0)	1 (10.0)	57 (26.9)	
30 – 39	18 (29.0)	12 (25.5)	2 (50.0)	10 (25.6)	18 (36.0)	0 (0.0)	60 (28.3)	
40 – 49	14 (22.6)	12 (25.5)	0 (0.0)	9 (23.0)	4 (8.0)	2 (20.0)	41(19.3)	
> 50	11 (17.7)	4 (8.5)	1 (25.0)	3 (7.7)	7 (14.0)	2 (20.0)	28 (13.2)	
Gender								
Male	38 (61.3)	22 (46.8)	2 (50.0)	23 (59.0)	28 (56.0)	8 (80.0)	121 (57.1)	0.445
Female	24 (38.7)	25 (53.2)	2 (50.0)	16 (41.0)	22 (44.0)	2 (20.0)	91 (42.9)	
Residency								
Erbil	22 (35.5)	18 (38.3)	3 (75.0)	14 (35.9)	10 (20.0)	7 (70.0)	74 (34.9)	0.001
Duhok	5 (8.0)	10 (21.3)	1 (25.0)	11 (28.2)	11 (22.0)	1 (10.0)	36 (17.0)	
Sulaimania	13 (21.0)	8 (.17.0)	0 (0.0)	6 (15.4)	7 (14.0)	2 (20.0)	39 (18.4)	
Kirkuk	13 (21.0)	2 (4.3)	0 (0.0)	4 (10.2)	8 (16.0)	0 (0.0)	27(12.7)	
Nainawa	6 (9.7)	4 (8.5)	0 (0.0)	3 (7.7)	9 (18.0)	0 (0.0)	22 (10.4)	
Anbar	3 (4.8)	5 (10.6)	0 (0.0)	1 (2.6)	5 (10.0)	0 (0.0)	14 (6.6)	
Total	62 (29.2)	47 (22.7)	4 (1.9)	39 (18.4)	50 (23.6)	10 (4.7)	212 (100.0)	

* Total of all genotypes in that category
** One Way ANOV

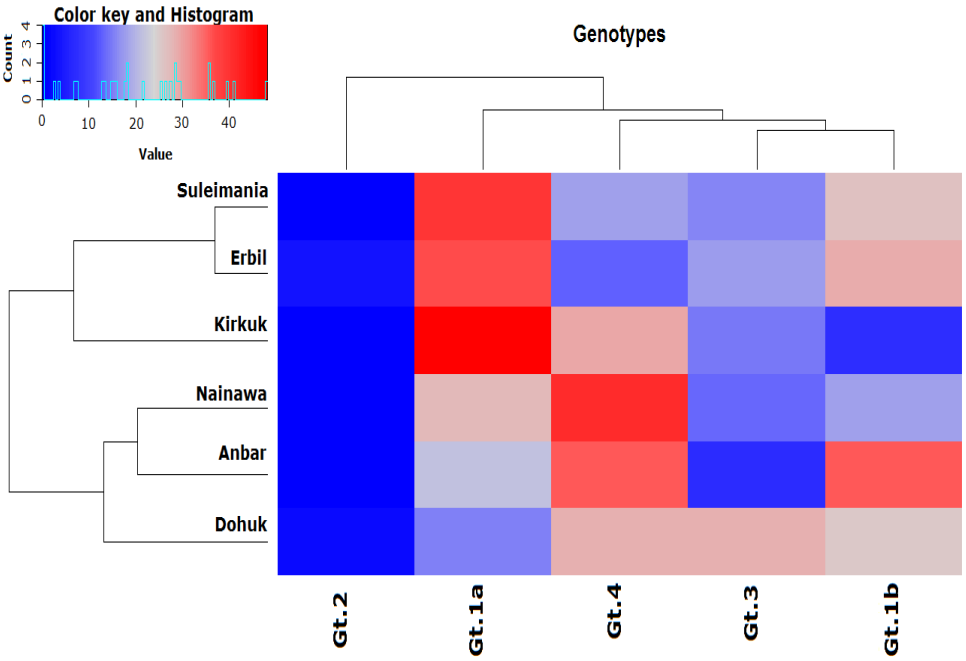

Figure 2: Heat map and combined dendrograms of HCV genotypes based on their counts and geographic distribution. The length of hierarchical clusters represents the distance between groups

Degree of Similarity for Geographic Distribution and Frequency of Genotypes: The heat map and dendrogram demonstrated the highest similarity between Erbil and Sulaimania compared to other provinces and the highest similarity between genotype 1b with genotype 3 then genotype 4 with regard to counts and distribution, (Figure 2).

Genotype Distribution and Viral Load According to Age of the Patients: Various genotypes had no significant different viral loads with regard to the age of the patients (P= 0.86), while the quantity of viral load was very weakly correlated with the increasing age (R^2 = 0.006) (Figure 3).

Viral Load Distribution According to Genotypes: The mean viral load values were between 10^7 – 10^8 IU/ml for all genotypes except genotype 2. Nevertheless, the overall distribution of viral load among genotypes showed no statistically significant variations (P= 0.056) (Figure 4).

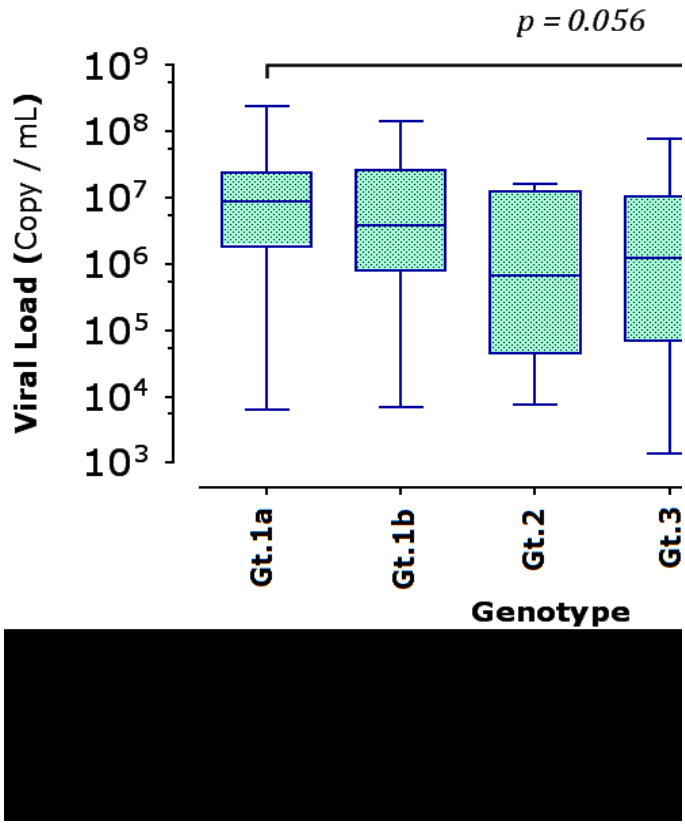

Figure 3: Genotypes distribution to the age of the patients (A). Correlation of the viral load to the age of the patient (B)

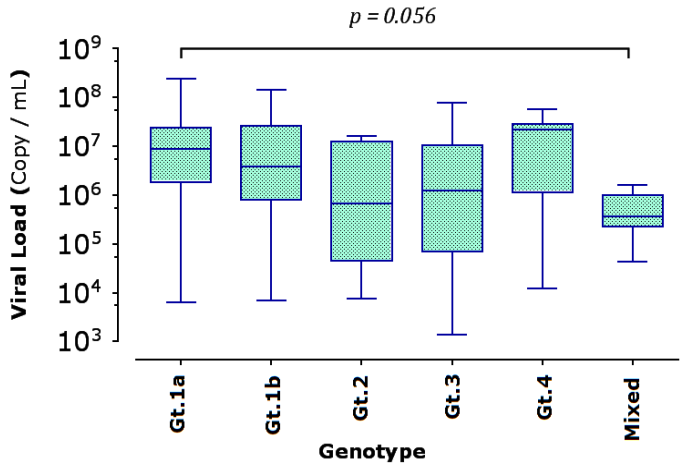

Figure 4: Box and whiskers demonstrating Viral load distribution according to genotypes. Error bars represent minimum and maximum

DISCUSSION

In reality, HCV genotyping is a helpful epidemiological marker, especially in identifying improbable vertical, intraspousal, or interfamilial HCV transmission paths[16,17]. Access to reliable assays of measuring viral load is of great importance to clinicians. The pretreatment of HCV RNA levels in patients with chronic HCV can be applied for prediction of therapeutic responses to interferon therapy with the help of HCV genotyping[18]. This study revealed that genotype 1a, genotype 4 and genotype 1b had the highest prevalence among Iraqi HCV infected patients; yet, different genotype distribution patterns were observed in the population of six governorates. Almost the same results were reported in a stud in Bahrain, where type 1 (36.71%) was the most common genotype among Bahraini patients, followed by types 3 and 4 (15.6% each)[19-21]. Additionally, our results supported those of a Pakistani study conducted on HCV-positive individuals in six cities in the Sindh province[2]. A systemic review and meta-analysis of data from different Eastern Mediterranean countries showed that genotype 4 was the most prevalent genotype in Egypt[22] and Saudi Arabia[23], with a rate of 69% and 65%, While genotype 1 was more prevalent in Iraq, Lebanon, Jordan, and the United Arab Emirates[26], subtype 1b was the most prevalent subtype in both Morocco[24] and Tunisia[25]. While a study from Erbil revealed that genotype 1 was the predominant genotype, another study in neighboring province of Dohuk stated that genotype 4 was the common one[27]. This diversity in the distribution pattern of genotypes in Iraq within the governorates compared to other countries could be due to secular variation in HCV distribution and a number of prevailing risk factors such as political conflicts, security problems, and internal displacement of the people, in addition to consequent economic and social factors.Age-related analysis of HCV revealed no significant statistical difference in the genotype distribution, where genotype 1a and 4 was more prevalent among young age groups (those less than 39 years), while genotype 4 was more prevalent among those aged 40 years and more. This is in contrast to the Pakistani study, where the highest rate of genotype 3a was reported among the same age group[2]. Also, a study in China revealed that genotype 1 was most common in male patients of the age group 50–59 years followed by 20–29 years[28-30]. On the other hand, a study in Italy showed no significant difference in age distribution of HCV genotypes[31], while there was a limited exposure among younger age groups according to a study in Saudi Arabia[32]. However, a higher frequency of genotype distribution among younger age groups in our community could be due to the fact that younger generation is more serious about their health and had a better understanding of the consequences of the disease compared to older age groups. Our study showed no significant difference between genotype distributions in regards to patients' gender and significant difference to patient's residency, which is in contrast to the findings of related studies in Pakistan and China[2,33], but almost similar to the results of a study in Saudi Arabia [32]. In addition, a study in Kenya found a significant (P= 0.031) association between residence and HCV infection, with much greater frequency among residents of the Southern Provinces[33].The overall viral load of one million IU/ml and higher was more among males with no significant statistical association, a study in China revealed that the overall viral load in males was higher than in females (P=0.006)[34]. This increased infection rate in men may be caused by the female body's natural tendency to naturally clear acute illnesses. The overall viral load of one million IU/ml and higher was more among younger age groups with no significant statistical correlation. This is in agreement with a study in the USA, which revealed that the HCV load did not correlate with age or sex for either group of patients[35,36], also; it is in contrast to another study in the USA that showed age and gender of the patients are correlated with the viral load[37-39]. It has been noted that after an acute infection, women are more likely to experience a spontaneous viral clearance. As a result, men are still more likely than women to have HCV viremic chronic hepatitis[40-43]. In this study, the mean viral load values were between 10^7 – 10^8 IU/ml for all genotypes except the genotype 2 with no significant differences in the overall distribution of viral load among the genotypes. This is in contrast to studies in France and China, where HCV genotype was correlated with viral load[44,45].

CONCLUSION

The study had concluded that genotypes 1a, 4 and 1b were more prevalent among the HCV patients, especially those from Erbil, Kirkuk and Sulaimania. Age-related prevalence showed no significant

difference of genotype distribution among the age groups, with higher prevalence among males and significant diverse distribution of the genotypes across the North West governorates. The overall viral load of one million IU/ml and higher was observed among younger age groups and males with no significant correlation in the overall distribution of viral load among genotypes.

Authorship Contribution: All authors share equal effort contribution towards (1) substantial contributions to conception and design, acquisition, analysis and interpretation of data; (2) drafting the article and revising it critically for important intellectual content; and (3) final approval of the manuscript version to be published.

Potential Conflict of Interest: None

Competing Interest: None

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