Assessment of hepatitis C viral load and genotypes by molecular determination in Iraqi patients treated with Ledipasvir/sofosbuvir (Harvoni) drug

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Abstract
Hepatitis C virus (HCV) is a significant global health threat that is responsible for approximately 170 million chronic infections worldwide. A feasible research was conducted to provide more understanding of viral load, effectiveness of Harvoni drug on virus concentration, and distribution of virus genotypes in Iraqi patients. Ninety eight HCV cases were investigated in this research, including 52 untreated, with an average age ± SE of 45.26 ± 2.97 years, and 46 treated with Harvoni therapy, with an average age of 39.30 ± 3.90 years. In addition, eighty healthy persons with an average age of 29.40 ± 2.84 years were included as control. These cases were attending to the Special Nursing Home Hospital in Baghdad between December 2018 and January 2019. They were diagnosed with this disease by using a real-time PCR to concentrate the viral level and determine viral genotypes. Statistical analysis showed that high substantial variances in virus concentration between untreated and treated patients and between both groups versus apparently healthy volunteers group (p < 0.01). Furthermore, genotype 4 was shown to be higher in our sample of Iraqi hepatitis C patients in comparison with the other patterns.

Keywords: Hepatitis C virus; viral load; viral genotypes.

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Introduction

The hepatitis C virus is one of the leading causes of death in humans due to the devastating effects on the liver. Seven major patterns, with 30–35% variation at the nucleotide level, and 67 subtypes, with less than 15% difference at the nucleotide level, were reported for this virus. The virus was discovered in 1989 and is responsible for more than half of the cases of chronic hepatitis in the world [1]. The virus is transmitted by several ways, such as infected blood or blood products, injection drug use, risky sexual intercourse, and transmission from infected mothers to their newborn children, which has a limited epidemiological role because it constitutes less than 5% of the cases [2]. The infection is randomly spread in different regions of the world, with occurrence in the general population that varies from 0.5 to 1.5%; it reaches 2.3% in countries of south-east Asia and eastern Mediterranean regions [3]. The general spreading patterns of each genotype of HCV are different across various geographical regions. HCV genotype 1 accounts for 46% of all HCV infections and is the most prevalent in the world, followed by genotype 3 which constitutes 30% and is more common in south Asia, Australia, and some European countries. HCV genotypes 2 and 4 account for 9-13 percent of HCV infections, with a more restricted geographic dispersal; genotype 4 is highly occurring in the Mediterranean basin and Africa. Genotypes 5, 6, and 7 are the greatest restricted in geographical spreading [4].

Viral load is the amount of viruses present in a certain volume of blood taken from an infected person. More precisely, it means the amount of viral genetic material in the blood and, therefore, indicates the number of virus particles in the blood, also termed as viral titer or viral copies [5]. The World Health Organization (WHO), using international standards, reported a viral load of two million copies/ml, which is the predictive cut-off value of significance for therapeutic efficacy in initial clinical IFN trials, being found to resemble 800 000 IU/ml [6]. Approaches for reliable quantitative tests of serum and plasma HCV levels of RNA have become important resources for both understanding HCV infection biology and clinical management of patients under care. The ability to define patients responsiveness to treatment by measuring low levels of HCV provides a more accurate tool for the treatment management and the classification of irresponsible patients to therapy early in the treatment, which prevents disease progression and high cost [7]. There are two important viral indicators that are used to assess the progression of disease and determine the effectiveness of the drug used in the treatment of viral hepatitis; these are viral load and virus genotypes [8]. Therefore, the concentration of the virus in the sera of patients with viral hepatitis is an important indicator for assessing virus progression, determining the appropriate treatment, and monitoring the response to treatment.

Previously, the drug commonly used to treat viral hepatitis C was peglated interferon (alfa-2a or alfa-2b), but in the last five years, the treatment of this disease has developed rapidly, especially against the genotype 1 [9]. The US Food and Drug Administration approved the use of Harvoni (ledipasvir/sofosbuvir) in 2014, as a new drug for treating the patients with Hepatitis C virus genotype 1 [10]. Ledipasvir and sofosbuvir are both directly-acting antiviral agents. Regarding sofosbuvir, some adjustments were made to allow this drug to be used in the treatment of genotypes 1 and, 2 as this drug functions through hindering NS5B protein manufacturing [11]. As for the second component (Ledipasvir), it interferes with NS5A synthesis and is effective against 1a, 1b, 4a, and 5a, with lower effectiveness against genotypes 2a and 3a [12]. Accordingly, we noticed that this new treatment has heterogeneous activities against viral genotypes. Therefore, this study was conducted to investigate viral load and prevailing genotypes in Iraqi patients in response to this drug.
Materials and Methods

Patients and control

This research was conducted from December 2018 to January 2019. Ninety-eight asymptomatic patients with HCV were admitted to the Special Nursing Home Hospital in Baghdad. These patients were divided into two clinical subgroups: 52 untreated patients and 46 subjects who received Harvoni drug. Eighty apparently healthy volunteers were included in this study as a control group. The viral load and genotypes of hepatitis C virus were detected by Real-Time PCR.

HCV RNA extraction and cDNA synthesis

Viral genome was extracted from the patient and healthy control sera by Acrogene Kit. Five μl of viral RNA was used for the synthesis of cDNA by using TonkBio kit, as in the following. In addition to viral RNA, 1 μl oligodT primer and 6.5 μl RNase were added into sterile, nuclease-free tube on ice and incubated at 65 °C. The vial was placed on ice and all the components required for cDNA synthesis were added and incubated at 42 °C for 1 hour. The reaction was terminated by heating at 70 °C.

Viral load detection

Viral load was detected in the studied groups by using Sacace HCV Real-TM Quant Dx PCR Kit. Fifty μl of RNA sample, calibrator, negative control, and positive control were added to the reaction tubes which contain all the components required for DNA amplification. Then, the tubes were closed and transferred into the Real Time PCR instrument, with the temperature profile described in table 1.

| Table 1—Real time PCR temperature profile |
|-------------------------------------------|
| Stage | Temp. °C | Time     | Fluorescence detection | Cycle repeats |
|-------|----------|----------|------------------------|---------------|
| Hold  | 50       | 15 min.  | -                      | 1             |
| Hold  | 95       | 15 min.  | -                      | 1             |
| Cycling | 95      | 5s       | -                      | 5             |
| 60    | 20s      | -        |                        |
| 72    | 15s      | -        |                        |
| Cycling 2 | 60   | 30s | FAM,JOE/HEX/CY3   | 40            |
| 72    | 15s      | -        |                        |

Ten μl of internal control was introduced into each sample at the beginning of sample preparation procedure. Each DNA amplification step was associated with the generation of a fluorescence signal that is measurable in the FAM/Green channel (for IC) or the Joe/Yellow/HEX channel (for HCV RNA), resulting in a sigmoid growth curve (log scale). The linear range of the HCV Real-TM Quant Dx kit was determined by analyzing a dilution series (from 8,00 to 1,00 log IU/ml) of an HCV synthetic quantitative standard (Figure 1).

Figure 1—Standard curve obtained by Excicycler Real-Time PCR.
Genotypes
Sacace HCV Real-TM Qual Dx PCR Kit was used for the qualitative detection and differentiation of HCV genotypes. Four PCR tubes were prepared for each sample and marked properly (1b/3, 1a/2, 4/IC, 5a/6). Fifteen μl of Master Mix and ten μl of cDNA sample, negative control and positive control, and cDNA HCV genotypes 1b/3, 1a/2, 4/IC and 5a/6 were added to the mix tubes. The tubes were closed and transferred into the real time PCR instrument with the temperature profile shown in Table-2.

Table 2-Real Time PCR Temperature profile

| stage       | Temp.˚C | Time  | Fluorescence detection | Cycle repeat |
|-------------|---------|-------|------------------------|--------------|
| Hold        | 95      | 15 min.| -                      | 1            |
| cycling     | 95      | 5 s   | -                      | 5            |
|             | 60      | 20 s  | -                      |              |
|             | 72      | 15 s  | -                      |              |
| Cycling 2   | 95      | 5 s   | -                      |              |
|             | 60      | 30 s  | FAM, JOE/HEX/CY3       | 40           |
|             | 72      | 15 s  | -                      |              |

Data analysis
The fluorescence curves were analyzed with the software of Real Time PCR instruments on the 2 channels (FAM/Green and Joe/Yellow), as listed in table 3.

Table 3-Real Time PCR two channels (FAM/Green and Joe/Yellow).

| Tube         | 1b/3 | 1a/2 | 4/IC   | 5a/6 |
|--------------|------|------|--------|------|
| FAM (Green)  | 1b   | 1a   | IC     | 5a   |
| JOE (Yellow) | 3    | 2    | 4      | 6    |

Results and discussion
Table-4 shows the results of viral load in the untreated group which had a value of 8666350.09 IU/ml (34039170.27 copy/ml), while the value in the treated group was 171172.00 IU/ml (653518.00 copy/ml) and that in the control was 0.0 IU/ml. Statistical analysis revealed high significant differences in viral load values between the untreated and treated groups and between these two groups versus the control (p< 0.01).

Table 4-Comparison between different groups in terms of viral load

| Group            | V. load (IU/ml) Mean ± SE | V. load copy Mean ± SE |
|------------------|---------------------------|------------------------|
| Control          | -                         | -                      |
| With treatment   | 171172.00 ± 155572.00     | 653518.00 ± 653458.00  |
| Without treatment| 8666350.09 ± 3432852.10   | 34039170.27 ± 13796991.45 |
| LSD value        | 136872.08 **              | 327966.38 **           |
| P-value          | 0.0001                    | 0.0001                 |

Viral genotypes among studied group
Table 5 reveals that the percentage of positive hepatitis C virus subjects among the untreated patients group was 84.62% (44/52), treated patients group was 8.70% (4/46), and healthy control group was 0.0% (0/80). In addition, the outcomes of Real Time-polymerase chain reaction that are
shown in the same table reveal three patterns of positive HCV in the studied groups; percentage of
genotype 1 prevalence in the untreated patients group was 3.85% (2/52), treated patients group was
0.00% (0/46), and healthy control group was 0.00% (0/80). While genotype 3 proportion in the
untreated patient group was 3.85% (2/52), treated patients group was 0.00% (0/46), and healthy
control group was 0.00% (0/80). The genotype 4 was recorded in 76.92% (40/52) of untreated patients
group, 8.7% (4/46) of treated patients group, and 0.00% (0/80) in the control. Statistical analysis
revealed highly significant differences among the studied groups according to genotyping patterns (P<
0.01). In general, genotype 4 percentage was greater in hepatitis C patients in comparison with other
patterns.

Table 5-Distribution of sample according to genotype

| Genotype | Control No. (%) | With treatment No. (%) | Without treatment No. (%) | Chi-Square ($\chi^2$) |
|----------|-----------------|------------------------|---------------------------|----------------------|
|         |                 |                        |                           |                      |
| Negative | 80 (100%)       | 42 (91.30%)            | 8 (15.38%)                | 13.58 **             |
| 1        | 0 (0.00%)       | 0 (0.00%)              | 2 (3.85%)                 | 0.674 NS*            |
| 3        | 0 (0.00%)       | 0 (0.00%)              | 2 (3.85%)                 | 0.674 NS*            |
| 4        | 0 (0.00%)       | 4 (8.70%)              | 40 (76.92%)               | 12.52 **             |
| Total    | 80              | 46                     | 52                        | ---                  |

Chi-Square ($\chi^2$) 15.00 ** 14.62 ** 12.76 ** ---

NS*: non-significant

The present results agree with those of Abdul- Sada (2011) [13] and Yahya et al. (2013) [5]. The
former study reported that the viral load ranged from $10^2$ to $4.5 \times 10^6$ IU/ml of blood, whereas
the mean and median values were $5.8 \times 10^6$ and $3.6 \times 10^6$ IU /ml of blood, respectively. The latter study
recorded that the viral units ranged from $1.19 \times 10^5$ IU/ml to $4.3 \times 10^5$ IU/ml and the mean was
$5.9 \times 10^5$ IU/ml, whilst the median was $2.6 \times 10^5$ IU/ml of blood. The connection between the genotypes
of HCV and the viral load remains disputed. High titer viraemia was linked to advanced liver disease
stages in some studies [14], whereas others showed the association with HCV genotype [15].
This extensive range of viral load can be due to the diversity of patients from various groups, ages, and
disease phases. The immune response and the patient's recent status were also shown to play an
essential role in viral load oscillation in identical individuals [16, 17]. However, the present work
showed no variation in viral titer between patient groups, due to the large ratio of infections that
belonged to the similar genotype (Genotype 4).

The present study displays highly significant differences between treated and untreated groups in
viral load levels. These results support that Harvoni drug has potential effects in viral infection
treatment. Previously, the treatment that was commonly used to treat patients with hepatitis C virus
was composed of a combination of pegylated interferon (alfa-2a or alfa-2b) given by injection for two
weeks and weight-based dose of ribavirin given orally for 48 weeks. This treatment led to a sustained
viral response (SVR) rate of 45% -50% [9]. A previously conducted study showed that the use of
Harvoni as a therapy was more effective and resulted in an SVR rate of 97% or higher, while the
discontinuation was higher (for 24 weeks of treatment) versus 12 weeks in the old treatment. The
study showed that 12 weeks of lidipasvir / sofosbuvir without ribavirin is an effective treatment for
patients with hepatitis C virus genotype 1 infection [12]. The effectiveness of Harvoni may be
attributed to strong effects on the synthesis of the viral components, such as the inhibition of HCV
NS5B RNA-dependent RNA polymerase, which acts as a chain terminator [18]. Inhibition of
hyperphosphorylation of NS5A plays a critical role in stopping virus synthesis and preventing viral
assembly [19]. This effectiveness can be confirmed with highly sensitive analyses after 24 weeks of
management, because patients with measurable HCV RNA at this time point only have a 1-2% chance
of reaching sustained virological response (SVR). SVR is identified as the absence of noticeable HCV
RNA after 24 weeks of treatment. It must be estimated by an HCV RNA detection test with a
minimum threshold of 50 IU / ml or lower to evaluate long-term success of the treatment [20].
Knowledge of virus genotypes plays critical role in identifying the source of outbreak and infection,
which may be spread horizontally (person to person) or vertically (mother to baby), as well as through
sexual contact and needle injections [21]. In this study, in an Iraqi sample from Baghdad, the goal was
to limit the prevalence of HCV genotypes in patients infected with Hepatitis C virus in order to determine and improve direct acting antivirals (DAAs) for the patients. Three genotypes, namely 1, 3, and 4, were observed, but the latter was the most commonly found.

HCV genotype 1 is the most prevalent (46%) genotype globally and predominates in Europe, North America, and Australia, followed by Genotype 3 (30%) which is primarily distributed in South Asia, particularly among Indians [22]. Infections with HCV genotype 4 are mainly found in Africa and the Middle East [23]. The recent study is agreement with four previous local studies in the Iraqi population, primary that conducted by Al-Kubaisy et al. (2006) [24] which stated that genotype 4 was the highest recurrent type, followed by 1a and 1b, with the exhibition of a mixed genotype (1a and 4) infection. The second trial was conducted by Abdul-Sada (2011) [13] who showed that the predominant genotype 4 was existing in 89.4% of the infected patients, followed by groups 6a, 3a, 2b, and 1b with percentages of 1.94%, 2.91%, 2.91%, and 6.79% respectively. The third research was published by Yahya et al. (2013) [5] who indicated three HCV genotypes (1a, 1b and 4) where the genotype 4 was dominant (86.27%), followed by 1b (37.25%), and 1a (33.33%). Eventually, the study of Jawad et al. (2014) [25] exhibited that HCV genotype 4 is the main genotype (56%) followed by genotype 1b (20% three percent) and genotype 1a (twelve percent), while genotype 3 was found in only nine percent. The prevalence of genotype 4 in Iraq is confirmed by these reports as well as the current study. However, in Iraqi hemodiagnosis patients, the prevalence of HCV genotype 4 is in line with other studies conducted on the genotyping of HCV isolates in various Middle Eastern countries, such as Egypt (up to 80 percent) [26]. Genotype 4 epidemiological profile consists of settlers from other nations, mostly Egyptian migrants who were diagnosed with HCV-4. Owing to the usage of unsterile tools during mass treatment of people with parenteral antischistosomal from the 1920s to the 1980s, Egypt has the main prevalence of HCV in the region [27]. Around 90 percent of Egyptian patients are HCV-4 positive [26]. Genotype 1a epidemiology profile was related to injecting drug use (IDU), whereas genotype 1b is more frequently associated with patients who received HCV via blood transfusion [28]. The surprising levels of genotypes 1 and 3 in the present study can be ascribed partially to the open human movement policy adopted by the Iraqi government over the last 15 years, chiefly in the population of Baghdad city. Consequently, the return of large numbers of Iraqi people living in foreign countries, in addition to the influx of people of different ethnicities (Asians, Europeans, and Americans) may have contributed to this presence of 1 and 3 genotypes in Iraq.

Conclusions

Harvoni drug has very good effects in reducing the concentration of HCV which was present in three genotypes (1, 3 and 4), with genotype 4 being the most commonly encountered genotype.

References

1. Coppola, N., Minichini, C., Starace, M., Sagnelli, C. and Sagnelli, E. 2016. Clinical impact of the hepatitis c virus mutations in the era of directly acting antivirals. J. Med Virol. 88:1659–71.
2. Benova, L., Mohamoud, Y.A., Calvert, C. and Abu-Raddad, L.J. 2014. Vertical transmission of hepatitis C virus: systematic review and meta-analysis. J. Clin Infect Dis. 59:765–73.
3. World Health Organization. 2017. Global Hepatitis Report. http://apps.who.int/iris/bitstream.
4. Smith, D.B., Bukh, J., Kuiken, C., Muerhoff, A.S., Rice, C.M., Stapleton, J.T. and Simmonds, P. 2014. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource. J. Hepatology. 59:318–27.
5. Yahya, A. A., Adnan, H. A. and Bushra, J. H. 2013. Determination of Hepatitis C Viral Load and Genotypes by Real-Time and RT-PCR at Thi_Qar Province. QMJ 9:15.
6. Strader, D.B., Wright, T.L., Thomas, D.L. and Seeff, L.B. 2004. Diagnosis, management and treatment of hepatitis C. AASLD Practice guideline. J. Hepatol. 39:1147.
7. Carman, B. 2001. Molecular techniques should now replace cell culture in diagnostic virology laboratories. Rev. J. Med. Virol. 11: 347-349.
8. Florence, K., Gla’ucia, P., Mireille, S., Philippe, C., Bernard, M., Vincent, L., Patrice, A. 2001. Quantitation of HCV RNA using real-time PCR and fluorimetry. J. Virolological Methods 95:111–119.
9. Issur, M. and Gotte M. 2014. Resistance patterns associated with HCV NS5A inhibitors provide limited insight into drug binding. J. Viruses 6(11):4227–4241.
10. Food and Drug Administration. 2014. FDA approves first combination pill to treat hepatitis C. http://www.fda.gov/News Events/ Newsroom/ Press Announcements/ ucm418365. htm.
11. Sofia, M.J. 2014. Beyond sofosbuvir: What opportunity exists for a better nucleoside/ nucleotide to treat hepatitis C? J. Antiviral 107:119–124.
12. Diana, G. and Gregory, H. 2015. Ledipasvir/Sofosbuvir (Harvoni): Improving Options for Hepatitis C Virus Infection. J. P&T 40:4.
13. Abdul-Sada, K.M. 2011. Estimation of HCV genome genotyping and the role of mosquitoes in its transmission. Ph.D. Thesis, College of Medicine, University of Kufa. Iraq.
14. Adinolfi, L.E., Gambardella, M., Andreana, A., Tripodi, M.F., Utili, R. and Ruggiero, G. 2001. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. J. Hepatol., 33(6): 1358–64.
15. Shahraki, T., Shahraki, M., Moghaddam, E., Najafi, M. and Bahari, A. 2010. Determination of Hepatitis C Genotypes and viral titer Distribution in children and adolescents with Major Thalassemia. Iran J Pediatr., 20(1):75-81.
16. Santantonio, T., Wiegand, J. and Gerlach, J.T. 2008. Acute hepatitis C: current status and remaining challenges. J. Hepatol. 49(4):625-33.
17. Jacobson, I. M., Davis, G. L., El-Serag, H., Negro, F. and Trepo, C. 2010. Prevalence and challenges of liver diseases in patients with chronic hepatitis C virus infection. Clin. Gastroenterol. Hepatol., 8(11):924-33.
18. Sofia, M.J. 2014. Beyond sofosbuvir: What opportunity exists for a better nucleoside/ nucleotide to treat hepatitis C? J. Antiviral 107:119–124.
19. Pawlotsky J.M. 2013. NSS5A inhibitors in the treatment of hepatitis C. J. Hepatol 59(2):375–382.
20. Manns, M.P., Wedemeyer, H. and Cornberg, M. 2006. Treating viral hepatitis C: efficacy, side effects, and complications. Gut., 55(9):1350-9.
21. Pekova, L.M., Teocharov, P. and Sakarev, A. 2007. Clinical course and outcome of a nosocomial outbreak of hepatitis C in a urology ward. J. Hosp. Infect., 67(1):86-91.
22. Sergio, M. B., Charlotte, H., Bandita, P., Robert, H. H., Luisa, M. S., Diana, M. B., Mani, G. S., John, G. M., Hongmei, M. E. S. and Stephen, D. S. 2018. Identification of a Novel Hepatitis C Virus Genotype from Punjab, India - Expanding Classification of Hepatitis C Virus into 8 Genotypes. Downloaded from https://academic.oup.com.
23. Charlotte, H., Bandita, P., Silvia, C., Stefan, Z., Christophe, M., Stephen, D. S., Sergio, M. B., Tarik, A., Laurent, A., Armand, A., Jyh-Jou, C., Jane, C., Dharmesh, K., Robert, H. H., Peter, S., Hongmei, M. and Evgenia, S. S. 2019. Identification of 19 Novel Hepatitis C Virus Subtypes- Further Expanding HCV Classification Downloaded from https://academic.oup.com.
24. Al-Kubaisy, W.A., Al-Nabi, K.T. and Habib, M.A. 2006. Seroprevalence of hepatitis C virus specific antibodies among Iraqi children with thalassaemia. Eas. Mediterr. Heal. J., 12(1-2):204-10.
25. Jawad, K. M., Eman, S. A. and Omar, F. A. 2014. Distribution of Hepatitis C Virus (HCV) Genotype among Iraqi Hemodialysis Patients. J. Advanced Research 2(1):442-445.
26. Ray, S.C., Arthur, R.R., Carella, A., Bukh, J. and Thomas, D.L. 2000. Genetic epidemiology of hepatitis C virus throughout Egypt. Infect. Dis., 182:698–707.
27. Frank, C., Mohamed, M.K., Strickland, G.T., Lavanchy, D., Arthur, R.R., Magder, L.S., El Khoby, T., Abdel-Wahab, Y., Aly Ohn, E.S., Anwar, W. and Sallam, I. 2000. The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. Lancet., 355: 887–891.
28. Elghouzzi, M.H., Bouchardeau, F., Pillonel, J., Boiret, E., Tirtaine, C., Barlet, V., Moncharmont, P., Maisonneuve, P., Puy-Montbrun, M.C., Lyon-Caen, D. and Courouce, A.M. 2000. Hepatitis C virus: routes of infection and genotypes in a cohort of anti-HCV-positive French blood donors.Vox Sang., 79:138-44.