



HOSTED BY



ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtbOriginal article <http://dx.doi.org/10.1016/j.apjtb.2016.12.003>

The distribution of HCV in subjects attending hospitals in Duhok City, Iraq

Nawfal Rasheed Hussein^{1*}, Amer Abdullah Balatay², Alpay Turan Sezgin³, Rana Saadi Jawad⁴¹Department of Internal Medicine, College of Medicine, University of Duhok, Duhok, Iraq²Department of Pathology, College of Medicine, University of Duhok, Duhok, Iraq³Department of Cardiology, Acibadem Institutes, Adana, Turkey⁴Respiratory & Chest Diseases Center, Duhok, Iraq

ARTICLE INFO

Article history:

Received 2 Sep 2016

Received in revised form 8 Oct 2016

Accepted 20 Nov 2016

Available online 5 Dec 2016

Keywords:

HCV

Genotype

Iraq

Duhok

ABSTRACT

Objective: To investigate the prevalence of hepatitis C virus (HCV) and its genotypes in Duhok City, Kurdistan Region, Iraq.**Methods:** A cross sectional study was conducted to investigate the prevalence of HCV and its genotype. A total of 2 109 subjects, who attended the hospital for complaints other than hepatitis, were recruited in this study.**Results:** First, anti-HCV antibody positivity was examined by ELISA. About 5.2% (111/2 109) of our samples were tested positive for anti-HCV antibodies. To confirm the positivity, RT-PCR was performed. Amongst all samples, 2.8% (60/2 109) was positive by RT-PCR. Then, we genotyped all the RT-PCR positive samples, and it was found that 50.0% (30/60) of our samples were typed as HCV genotype 4, 43.3% (26/60) as genotype 1 and 6.7% (4/60) as genotype 3.**Conclusions:** The prevalence of HCV was higher than that was reported previously and genotype 4 was the most prevalent. Further population based study is required to investigate the prevalence of HCV.

1. Introduction

Hepatitis C virus (HCV) infection poses a major global health problem [1]. Worldwide, it is estimated that about 200 million people are infected with HCV [2]. Chronic HCV infection might be associated with increased mortality rate as the infection predisposes to the development of liver cirrhosis, hepatocellular carcinoma and liver failure [3]. Studies from regional countries showed that HCV prevalence was 1.1% in Yemen, less than 1.0% in Iran, 1.8% among young generation in Saudi Arabia, 4.0% among blood donors in Pakistan and 0.2% in Iraq [4–6].

HCV is of genetic heterogeneity that makes the development of vaccine extremely difficult [7]. HCV genotype has also been reported as an important predictor in determining sustained

virologic response in HCV patients with treatment showing greater efficacy in genotype 2 and 3 than in genotype 1. Genotype 4 is the most problematic to treat [8] though sustained virologic response has been achieved upto 70% in Middle East populations [8,9]. This justifies the study of HCV genetic diversity at country level. The genotypes of HCV were studied previously in Kurdistan Region in patients with haemoglobinopathy and renal transplant subjects. HCV genotype 4 was the most prevalent genotype followed by genotype 1 [9–11]. The aims of this study were to investigate the prevalence of HCV positivity in patients attending the hospitals in Duhok City and then to study the genotype of HCV positive samples.

2. Materials and methods

2.1. Blood samples

Blood samples were collected randomly from 2 109 subjects visiting Azadi and Heevi hospitals for different reasons in the period from May 2015 to December 2015. A 5 cc syringe and needle was used to collect approximate 5 mL of blood from each donor. Then, the blood samples were centrifuged at 1 500 r/min for 3 min to obtain sera.

*Corresponding author: Nawfal Rasheed Hussein, Department of Internal Medicine, College of Medicine, University of Duhok, Duhok, Iraq.

Tel: +964 627244601

E-mail: nawfal.hussein@yahoo.com

The study protocol was performed according to the Helsinki declaration and approved by the Ethics and Research Committee of Azadi and Heevi hospitals and the School of Medicine. Written informed consent was obtained from the participants of this study.

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

2.2. ELISA

The HCV antibody (HCV-AB) (fourth generation) was studied by commercial ELISA kit (DIA.PRO Diagnostic Bio-probes, Milan, Italy) following manufacturer's instruction.

2.3. RNA extraction

RNA extraction was conducted using QIAamp RNA extraction kit (Qiagen, Hamburg, Germany) according to manufacture instructions for automatic extraction in QIAcube extractor (Qiagen, Hamburg, Germany). The extracted RNA concentration was confirmed through measurement by Nanodrop ND-1000 (Nanodrop, USA).

2.4. Quantification of HCV RNA and HCV genotyping

Quantification of HCV RNA was performed by real-time quantitative PCR. This was conducted using the Qiagen Artus HCV RG RT-PCR kit (Qiagen, Hamburg, Germany). An aliquot of 20 μ L of purified sample was utilised in a total reaction volume of 50 μ L. Amplification reaction for each sample and standard was performed in duplicates. Amplification cycling was performed using the Rotor-Gene Q device (Qiagen, Hamburg, Germany). Data analysis was performed with the Rotor-Gene software according to the manufacturer's instructions. HCV genotyping was performed by reverse hybridization technique (NLM, Milan, Italy).

2.5. Ethical approval

The study protocol was performed according to the Helsinki declaration and approved by the Ethics and Research Committee of Azadi and Heevi hospitals and the School of Medicine. Written informed consent was obtained from the participants of this study.

3. Results

3.1. Patients

During the period from May 2015 to December 2015, we examined 2109 samples for HCV-AB. The average age of recruited subjects was (37.30 \pm 7.78) years. We examined samples taken from 779 females (with average age of 36.10 years) and 1330 males (with average age of 38.03 years).

3.2. HCV positivity

During the period of study, we examined 2109 samples. First, anti-HCV antibody positivity was examined. About 5.2% (111/2109) of our samples were tested positive for anti-HCV antibodies. Then, to discriminate between current infection and old resolved infection, RT-PCR was performed for all positive samples. Amongst all samples, 2.8% (60/2109) was positive by RT-PCR. About 56.7% (34/60) of the positive samples were female.

3.3. HCV genotype

We genotyped all the RT-PCR positive samples. It was found that 50.0% (30/60) of our samples were typed as HCV genotype 4, 43.3% (26/60) as genotype 1 and 6.7% (4/60) as genotype 3.

4. Discussion

HCV is a prevalent virus and a leading cause of mortality and morbidity worldwide [12]. Chronic HCV infection predisposes to the development of liver cirrhosis, hepatocellular cancer and liver failure [12]. The prevalence rate of HCV infection is decreasing in the developed countries; however, in developing countries such as Iraq, researchers still struggle to control the infection [12,13].

Different studies from neighbouring countries showed a variation in the prevalence rates of HCV ranging from 0.4% to 19.2% [14,15]. In this study, we found that 5.2% of the samples were HCV-AB positive. Then, all samples were sent to PCR to confirm the positivity. It was found that 2.8% of the samples were PCR positive. This was higher than what was found previously in Iraq where less than 0.5% of blood donors were positive for HCV-AB [6,16–18]. This difference in the prevalence rate might be explained in part by the difference in sample collection method and the techniques used in the diagnosis. Further population based studies are needed to explore this. HCV genotype was studied previously in Iraq. In a study conducted in the south of Iraq, it was found that 35.0% of the samples typed as genotype 4 while 50.0% of the recruited samples typed as genotype 1 [19]. In another study from Iraq recruiting patients with haemoglobinopathy who were infected with HCV, it was shown that 53.0% of the samples typed as HCV genotype 4, followed by 23.0% for genotype 1, 20.9% for genotype 3 and 2.3% for genotype 2. In a study conducted in Iran, it was shown that the majority of Iranian samples were HCV genotype 1 (47.0%) followed by genotype 3 (36.0%) [20]. Previous studies from the Arab Gulf area showed that genotype 4 was the most prevalent HCV genotype [21,22]. In this study, 50.0% of the samples were genotype 4, followed by 43.3% for genotype 1 and 6.7% for genotype 3. Historically, genotype 4 and 1 HCV has been considered the most difficult hepatitis C genotypes for treatment. It was previously reported in Iraq that, only 50.0% of those were responding to the classical treatment. Newer direct acting antivirals therapy is very expensive in Iraq, therefore, strict measurements should be taken to prevent the infection.

Our study also has limitations. We studied the prevalence of HCV in sample collected from patients rather than a random community sample, and this may have introduced bias. This might explain the higher prevalence rate found in our study. A population based study is recommended to investigate the prevalence of HCV in this society. We believe that the prevalence of HCV might be very low in general population which might make studying the HCV genotype extremely difficult because of the low positivity.

Conflict of interest statement

We declare that we have no conflict of interest.

References

- [1] Flichman DM, Blejer JL, Livellara BI, Re VE, Bartoli S, Bustos JA, et al. Prevalence and trends of markers of hepatitis B virus, hepatitis C virus and human immunodeficiency virus in Argentine blood donors. *BMC Infect Dis* 2014; **14**(1): 218.
- [2] Zaheer H, Saeed U, Waheed Y, Karimi S, Waheed U. Prevalence and trends of hepatitis B, hepatitis C and human immunodeficiency

- viruses among blood donors in Islamabad, Pakistan 2005–2013. *J Blood Disord Transf* 2014; **5**: 217.
- [3] Walter SR, Thein HH, Amin J, Gidding HF, Ward K, Law MG, et al. Trends in mortality after diagnosis of hepatitis B or C infection: 1992–2006. *J Hepatol* 2011; **54**(5): 879–86.
- [4] Afzal MS, Ahmed T, Zaidi NU. Comparison of HCV prevalence in Pakistan and Iran; an insight into future. *Hepat Mon* 2014; **14**(1): e11466.
- [5] Kafi-Abad SA, Rezvan H, Abolghasemi H. Trends in prevalence of hepatitis B virus infection among Iranian blood donors, 1998–2007. *Transfus Med* 2009; **19**(4): 189–94.
- [6] Hussein NR, Haj SM, Almizori LA, Taha AA. The prevalence of hepatitis B and C viruses among blood donors attending blood bank in Duhok, Kurdistan Region, Iraq. *Int J Infect* 2017; **4**(1): e39008.
- [7] Te HS, Jensen DM. Epidemiology of hepatitis B and C viruses: a global overview. *Clin Liver Dis* 2010; **14**(1): 1–21.
- [8] Legrand-Abravanel F, Colson P, Leguillou-Guillemette H, Alric L, Ravaux I, Lunel-Fabiani F, et al. Influence of the HCV subtype on the virological response to pegylated interferon and ribavirin therapy. *J Med Virol* 2009; **81**(12): 2029–35.
- [9] Hussein NR, Tunjel I, Basharat Z, Taha A, Irving W. The treatment of HCV in patients with haemoglobinopathy in Kurdistan Region, Iraq: a single centre experience. *Epidemiol Infect* 2016; **144**(8): 1634–40.
- [10] Hussein NR, Saleem ZS. Successful treatment of hepatitis C virus genotype 4 in renal transplant recipients with direct-acting antiviral agents. *Am J Transpl* 2016; **16**(7): 2237–8.
- [11] Hussein NR, Saleem ZSM. Successful treatment of hepatitis C virus infection in a renal transplant patient with ribavirin and sofosbuvir: a case report. *Int J Case Rep Imag* 2016; **7**(6): 370–3.
- [12] Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. *Hepatology* 2013; **57**(4): 1333–42.
- [13] Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, et al. Global distribution and prevalence of hepatitis C virus genotypes. *Hepatology* 2015; **61**(1): 77–87.
- [14] Klevens RM, Hu DJ, Jiles R, Holmberg SD. Evolving epidemiology of hepatitis C virus in the United States. *Clin Infect Dis* 2012; **55**(Suppl 1): S3–9.
- [15] Vriend HJ, Van Veen MG, Prins M, Urbanus AT, Boot HJ, Op De Coul EL. Hepatitis C virus prevalence in The Netherlands: migrants account for most infections. *Epidemiol Infect* 2013; **141**(6): 1310–7.
- [16] Hussein N, Taher A. The seroprevalence of HBV and HCV infection in newly recruited police in Duhok City, Kurdistan Region, Iraq. *Int J Sci Basic Appl Res* 2015; **24**(1): 112–6.
- [17] Hussein NR. Prevalence of HBV, HCV and HIV and anti-HBs antibodies positivity in healthcare workers in Departments of Surgery in Duhok City, Kurdistan Region, Iraq. *Int J Pure Appl Sci Technol* 2015; **26**(2): 70–5.
- [18] Al-Juboury AM, Salih H, Assadi MK, Ali AM. Seroprevalence of hepatitis B and C among blood donors in Babylon Governorate, Iraq. *Med J Babylon* 2010; **7**(1–2): 121–9.
- [19] 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): 204–10.
- [20] Samimi-Rad K, Nategh R, Malekzadeh R, Norder H, Magnus L. Molecular epidemiology of hepatitis C virus in Iran as reflected by phylogenetic analysis of the NS5B region. *J Med Virol* 2004; **74**(2): 246–52.
- [21] Koshy A, Madda JP, Marcellin P, Martinot M. Treatment of hepatitis C virus genotype 4 related cirrhosis: ribavirin and interferon combination compared with interferon alone. *J Clin Gastroenterol* 2002; **35**(1): 82–5.
- [22] Ohno T, Mizokami M, Saleh MG, Orito E, Ohba KI, Wu RR, et al. Usefulness and limitation of phylogenetic analysis for hepatitis C virus core region: application to isolates from Egyptian and Yemeni patients. *Arch Virol* 1996; **141**(6): 1101–13.