Original Research Article

Prevalence and genotyping of Hepatitis C Virus in hemodialysis patients and evaluation of HCV-Core Antigen Test in screen Dialysis Patients in Sana'a city, Yemen

Abstract

Hepatitis C virus infection is a constant worldwide public health concern. The prevalence of HCV infection is much higher in patients on chronic haemodialysis (HD) than in the general population. HCV infection can detrimentally affect patients throughout the spectrum of chronic kidney disease. Despite the control of blood products, hepatitis C virus transmission is still being observed among patients undergoing dialysis. Detection systems for serum HCV antibodies are insensitive in the acute phase because of the long serological window. Direct detection of HCV depends on PCR test but this test is not suitable for routine screening. The objective of this study was to determine prevalence of HCV, genotyping and if HCV core antigen test could be better an alternative to NAT techniques for the diagnosis of HCV infection during the window period and whether the sensitivity for antibody detection is preserved. We screened 159 patients on long-term dialysis by HVC antibodies test, PCR HCV-RNA and HCV core antigen test by commercial tests.

The prevalence of HCV was 10.7% (17 patients) and genotype 4 was the most common one (64.7%). The sensitivity of HCV core antigen test was 94.1%, the specificity 100%, the positive predictive power 100%, and the negative predictive power 97.9%. In conclusions; patients on maintenance HD in Yemen have a high prevalence of HCV infection and genotype 4 is predominant. The performance of serological detection of HCV core antigen was better than that of HCV antibodies test and may be an alternative to nucleic acid amplification technology (NAT) for routine monitoring of patients on chronic dialysis.

Keywords: Haemodialysis, Hepatitis C virus, Genotype, HCV core antigen, HCV antibodies, PCR HCV-RNA, Yemen.

Introduction

An estimated 143 million people (2%) worldwide are infected with hepatitis C as reported for 2015.[1] In 2013 about 11 million new cases occurred.[1] It occurs most commonly in Africa and Central and East Asia.[2] About 167,000 deaths due to liver cancer and 326,000 deaths due to cirrhosis occurred in 2015 due to hepatitis C.[2]

In Yemen HCV antibodies among general community showed a steady decline to less than 0.5%[3,4], however among HCV risk groups as dental workers and public health center workers were 5.5% and 11.5% respectively.[5,6] Limited information is available in dialysis patients in Yemen. HCV infection is more common in dialysis patients than in healthy populations worldwide. The Dialysis Outcomes and Practice Patterns Study (DOPPS) reported an in general prevalence of 13.5 percent among adult hemodialysis patients randomly selected from 308 dialysis services in developed countries (France, Germany, Italy, Japan, Spain, the United Kingdom, and the United States).[7] A study from Australia and New Zealand[8] in HD patients (*n* = 23,046) reported an independent and significant association between anti-HCV positive serologic status and all-cause mortality over a 10-year follow up (HR, 1.25, 95% CI

1.07–1.46, $P = 0.004$). Regardless of the control of blood products, HCV transmission is stationary being observed among HD patients. HCV infection is usually diagnosed rooted in the detection of anti-HCV antibody, while it goes undetected in the first 4–6 weeks of infection (so-called window period). Furthermore, patients positive for anti-HCV antibody include both those who are actively infected and those who have recovered from infection.[9] Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guidelines for the prevention, diagnosis, evaluation, and treatment of hepatitis C in chronic kidney disease[10] recommended the use of nucleic acid amplification technology (NAT). A quantitative HCV core antigen (HCVcAg) test has been developed for the confirmation of viremia in patients with hepatitis C. This test can detect total nucleo-capsid core antigen whose sequence is highly conserved across HCV genotypes. A number of studies in the general population have highlighted the importance of HCV core antigen detection as an alternative to NAT for early diagnosis of infection, as direct marker of viral replication in chronic phase of infection and as relevant marker for predicting and monitoring the response to therapy.[11] Few studies exist about the efficacy of HCV core antigen test in patients on chronic HD in the early diagnosis of HCV infection.[12-14] The objective of this study was to determine prevalence of HCV, genotyping and if HCV core antigen test could be better an alternative to NAT techniques for the diagnosis of HCV infection during the window period and whether the sensitivity for antibody detection is preserved.

Subjects and Methods

The study was performed in haemodialysis units of Al-Thorah hospital haemodialysis unit. A total of 159 patients were enrolled in this cross sectional study in 2016; patients gave informed consent and thus the whole patient population were investigated. All patients underwent chronic haemodialysis treatment for end stage renal disease during the study period. The laboratory works conducted in Al-Awalagy Medical laboratory. Anti-HCV antibody was measured by a third generation commercial ELISA (Enzymun-Test Anti-HCV; Boehringer Mannheim, Germany). The third generation assay detects antibodies for three viral antigens (c22-3, c200, and NS5). HCV-RNA and viral genotype by PCR (TaqMan Roche). All tests were carried out and interpreted strictly in accordance with the manufacturer's instructions.

Statistical Analysis

The performance of the HCV core antigens and antibodies HCV test were done by comparing to HCV-RNA PCR test. The following parameters were calculated: sensitivity%, Specificity%, false positive% (FP), false negative% (FN), positive predictive value (PPV); and negative predictive value (NPV). To gender and age group's possible associated risk factors for HCV infection, the data were examined in a case-control study format. For HCV, persons with evidence of previous or current infection with HCV were matched up with those who were HCV negative.

Ethical Consideration

Ethical clearance for the study was taken from the Faculty of Medicine and Health Sciences Research Review Committee. Informed Consent was taken from the volunteers before the collecting specimens and file questionnaire.

RESULTS

Table 1shows the prevalence rate of HCV ribonucleic acid (RNA), and associated odds ratio for different sex and age of a sample of hemodialysis patients in Sana'a city. The crude prevalence rate of HCV among HD patients was 10.7%, for male patients was 14.3%, higher than 8.7% of female patients. When age was considered, there was rise trend of HCV infection with increasing age. The genotype distribution in 17 HCV-positive patients is shown in Table 2. Overall, HCV genotype 4 was the most predominant genotype (64.7%) followed by genotype 1a and 1b (29.4%) and 2a (5.9%). Table 3 shows the prevalence rate of different HCV markers in 159 haemodialysis patients in Sana'a city. A 17 HD patients were HCV ribonucleic acid (RNA) positive, 14 HD patients were HCV antibodies positive and 16 HD patients were HCV core proteins positive. Table 4 shows the performance of HCV antibodies test and HCV Core protein test comparing to HCV ribonucleic acid (RNA) among haemodialysis patients in Sana'a city. The sensitivity of HCV core antigen test was 94.1%, the specificity 100%, the positive predictive power 100%, the negative predictive power 97.9%, false positive rate 0.0% and false negative rate 5.9%. However less reliable results were found for HCV antibodies test in which the sensitivity of HCV antibodies test was 70.6%, the specificity 98.9%, the positive predictive power 100%, the negative predictive power 97.9% false positive rate 1.41% and false negative rate 29.4%. Thus 1.41% (FP) of the entire study population was HCV positive but could not be diagnosed by routine HCV antibody testing, while 0% (FP) of the entire study population was HCV positive could not be diagnosed by routine HCV core antigen test.

Discussion

Information of the prevalence of HCV in general population and in the various high risk groups as HD patients, prevalence of genotypes and evolution of tests used for screening HCV are important in prevention and control HBV infections. Unfortunately, there is little information available on these topics, particularly from Middle East countries and more specifically from Yemen. HCV infection continues to be the most frequently recognized cause of liver damage in patients with CKD.[15] Although a severe clinical course of HCV-related liver disease seems unusual in most HD patients and cirrhosis is an infrequent event among dialysis patients, longitudinal studies have found an independent and significant relationship between anti-HCV antibody positivity and reduced patient survival.[16,17] The Dialysis Outcomes and Practice Patterns Study (DOPPS) on HD patients in three continents [18] had reported an independent and significant association between positive anti-HCV antibody and mortality risk (adjusted relative risk, 1.17; $P < 0.0159$). Fabrizi et al.[19] showed that HCV-seropositive HD patients had higher rates of liver disease-related death than their sero-negative matching parts, but that cardiovascular and infectious disease related mortality rates were similar.[19] Ohsawa et al. showed that seropositivity for anti-HCVcAg is independently associated with increased all-cause, cardiovascular, and liver disease related mortality in HD patients.[20] In the current study in Yemen the crude prevalence rate of HCV among HD patients was 10.7%, (male rate=14.3%, female rate= 8.7%). This result is indicated that HCV infection is more common in dialysis patients in Yemen than in healthy populations (0.5-5%).[3-5] The current result also is slightly lower than the Dialysis Outcomes and Practice Patterns Study (DOPPS) which it reported an overall prevalence of 13.5% among adult hemodialysis patients randomly selected from 308 dialysis facilities in developed countries.[7]

In addition, our study results show that HCV genotype 4 is the predominant genotype (64.7%) among Yemeni patients followed by 1a and 1b (29.4%) and 2a (5.9%). This data is similar to that reported in Middle East countries, and previously in the Yemen; where genotype 4 is the predominant.[4, 21-24]

It is important to diagnose a hepatitis C virus infection in the acute phase in order to reduce the incidence of this infection in high-risk populations like HD patients.[25] Biochemical evaluation of HCV infection in patients with CKD is inaccurate. Serum aminotransferase values are typically lower in dialysis patients than the non-uremic populations.[26] Thus we carried out this study to evaluated the performance of HCV antibodies test and HCV Core protein test comparing to HCV ribonucleic acid (RNA) among our haemodialysis patients (table 4). The sensitivity of HCV core antigen test was 94.1%, the specificity 100%, the positive predictive power 100%, the negative predictive power 97.9%, false positive rate 0.0% and false negative rate 5.9%. However less reliable results were found for HCV antibodies test in which the sensitivity of HCV antibodies test was 70.6%, the specificity 98.9%, the positive predictive power 100%, the negative predictive power 97.9% false positive rate 1.41% and false negative rate 29.4%. Our results is similar to that reported previously in which detection systems for serum HCV antibodies are insensitive in the acute phase because of the long serological window.[7] Also, the direct detection of HCV depends on NAT techniques with several problems: frequent unavailability, considerable skill requirement, limited reproducibility, and overall important costs. HCV detection by PCR-RNA, although widely accepted as a gold standard test in the diagnosis of HCV infection in CKD patients, is not suitable for routine screening.[7] Thus from our results HCV core antigen quantification assay is proved useful for an early diagnosis of HCV infection in community-based and in dialysis populations. Also, HCV core antigen may be an alternative to HCV-RNA detection, since no subjects, who were negative for HCV core antigen, were positive for HCV-RNA (false negative=0.0%). Our result is similar to that reported in a large population-based cohort studies by Ohsawa et al. [27]; and by Kato et al. [28] in which no subjects, who were negative for HCV core antigen, were positive for HCV-RNA; also Ohsawa et al.[27] and Kato et al. [28] suggests that detection of HCV core antigen combined with anti-HCV antibody is useful in predicting long-term survival prognosis of persistent HCV infection in HD patients.

Finally, from our experience HCV core antigen test is both a cost-effective (a single sample has a 40$ charge for PCR HCVRNA and a 5$ charge for HCV core antigen test) and a less labour-intensive alternative to NAT tests. These features make this assay useful for routine of chronic dialysis treatment patients.

Conclusion

Patients on maintenance HD in Yemen have a high incidence and prevalence of HCV infection and genotype 4 is the predominant one. Serological detection of HCV core antigen may be an alternative to NAT techniques for routine monitoring of patients on chronic dialysis towards the prevention of HCV spread. HCV core antigen is an accurate marker for early identification of HCV infection; it can improve virological monitoring and integrate the diagnosis of acute hepatitis C in dialysis population. The minimal cost and its easiness make this assay useful for routine long-term dialysis treatment patients. Furthermore, screening for HCV antibodies alone does not exclude infection with HCV.

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Conflict of interest:

"No conflict of interest associated with this work".

Author's contribution

This research work is part of A MSc. thesis. The candidate is the third authorr (MSB) who conducted the laboratory and field works; and wrote up the thesis. The corresponding author (HAA) supervised the laboratory and field works, revised and edited the thesis draft and the manuscript and SHH revised and edited the thesis and the article.

References

1- GBD. "Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015". Lancet 2016; 388 (10053): 1545–1602.

2-WHO. "Hepatitis C Fact sheet N°164". WHO. July 2015. Archived from the original on 31 January 2016.

3-Al-Nabehi BAH, Al-Shamahy H, WSE Saeed, AM Musa, AM El Hassan,. Sero-molecular epidemiology and risk factors of viral hepatitis in Urban Yemen. Int J Virol 2015; 11 (3), 133-138.

4- Al-Shamahy HA, Abdu SSA. Genotyping of Hepatitis C Virus (HCV) in infected patients from Yemen. Eur J Basic Med Sci 2014;3 (4):78-82.

5- Al-Kebsi, A., A. Othman, A.-K. Abbas, E. Madar, H. Al-Shamahy, K. Al-Gaffari, S. M. Daname, and F. Motareb. Sero-prevalence of hepatitis c virus among dental clinic workers in Sana'a city-Yemen and the risk factors contributing for its infection Universal Journal of Pharmaceutical Research. 2017; 2(5): 28-33.

6- Al-Marrani Waleed Hasan Mohammed, Al-Shamahy Hassan A. Prevalence of HBV and HCV; and their associated risk factors among public health center cleaners at selected Public Health Centers in Sana'a city-Yemen. Universal Journal of Pharmaceutical Research. 2018; 3(5): 63-67.

7- Michel Jadoul a Paul Martin b. Hepatitis C Treatment in Chronic Kidney Disease Patients: The Kidney Disease Improving Global Outcomes Perspective. Blood Purif 2017;43:206–209.

8. Scott DR, Wong JKW, Spicer TS, *et al.* Adverse impact of hepatitis C virus infection on renal replacement therapy and renal transplant patients in Australia and New Zealand. Transplantation. 2010;90(11):1165–1171.

9. Laperche S, Le Marrec N, Girault A, *et al*. Simultaneous detection of hepatitis C virus (HCV) core antigen and anti-HCV antibodies improves the early detection of HCV infection. Journal of Clinical Microbiology. 2005;43(8):3877–3883.

10. KDIGO clinical practice guidelines for the prevention, diagnosis, evaluation, and treatment of hepatitis C in chronic kidney disease. Kidney International. 2008;73:S1–S99.

11. Veillon P, Payan C, Picchio G, Maniez-Montreuil M, Guntz P, Lunel F. Comparative evaluation of the total hepatitis C virus core antigen, branched-DNA, and amplicor monitor assays in determining viremia for patients with chronic hepatitis C during interferon plus ribavirin combination therapy. Journal of Clinical Microbiology. 2003;41(7):3212–3220.

12-Fabrizi F, Lunghi G, Aucella F, *et al*. Novel assay using total hepatitis C virus (HCV) core antigen quantification for diagnosis of HCV infection in dialysis patients. Journal of Clinical Microbiology. 2005;43(1):414–420.

13-Bouzgarrou N, Fodha I, Ben Othman S, *et al*. Evaluation of a total core antigen assay for the diagnosis of hepatitis C virus infection in hemodialysis patients. Journal of Medical Virology. 2005;77(4):502–508.

14-Miedouge M, Saune K, Kamar N, Rieu M, Rostaing L, Izopet J. Analytical evaluation of HCV core antigen and interest for HCV screening in haemodialysis patients. Journal of Clinical Virology. 2010;48(1):18–21.

15. Stehman-Breen CO, Emerson S, Gretch D, Johnson RJ. Risk of death among chronic dialysis patients infected with hepatitis C virus. American Journal of Kidney Diseases. 1998;32(4):629–634.

16. Nakayama E, Akiba T, Marumo F, Sato C. Prognosis of anti-hepatitis C virus antibody-positive patients on regular hemodialysis therapy. Journal of the American Society of Nephrology. 2000;11(10):1896–1902.

17. Espinosa M, Martin-Malo A, De Lara MAA, Aljama P. Risk of death and liver cirrhosis in anti-HCV-positive long-term haemodialysis patients. Nephrology Dialysis Transplantation. 2001;16(8):1669–1674.

18. Goodkin DA, Bragg-Gresham JL, Koenig KG, *et al.* Association of comorbid conditions and mortality in hemodialysis patients in Europe, Japan, and the United States: the Dialysis Outcomes and Practice Patterns Study (DOPPS). Journal of the American Society of Nephrology. 2003;14(12):3270–3277.

19. Fabrizi F, Takkouche B, Lunghi G, Dixit V, Messa P, Martin P. The impact of hepatitis C virus infection on survival in dialysis patients: meta-analysis of observational studies. Journal of Viral Hepatitis 2007;14(10):697–703.

20. Ohsawa M, Kato K, Tanno K, *et al.* Seropositivity for anti-HCV core antigen is independently associated with increased all-cause, cardiovascular, and liver disease-

related mortality in hemodialysis patients. Journal of Epidemiology. 2011;21(6):491–499.

21-Osaba AO. Hepatitis C virus genotypes in Saudi Arabia. Saudi Med 2002. J, 23(1): 7-12.

22-Marie Mohammed Ali M. Genotyping of Hepatitis C virus (HCV) in infected patients from Saudi Arabia. African Journal of Microbiology Research 2011, 5(16): 2388-2390.

23-Bdour S. Hepatitis C virus infection in Jordanian hemodialysis units, serological diagnosis and genotyping. J. Med. Microbiol 2002, 51(8):700-4.

24-Al Balwi MA. Prevalence of mixed hepatitis C virus (HCV) genotypes among recently diagnosed dialysis patients with HCV infection. Saudi Journal of Kidney Diseases and Transplantation 2011; 22(4):712–6.

25. Di Napoli A, Pezzotti P, Di Lallo D, Petrosillo N, Trivelloni C, Di Giulio S. Epidemiology of hepatitis C virus among long-term dialysis patients: a 9-year study in an Italian region. American Journal of Kidney Diseases. 2006;48(4):629–637.

26. Fabrizi F, Dixit V, Messa P, Martin P. Hepatitis C- related liver disease in dialysis patients. In: Morales JM, editor. Hepatitis C in Renal Disease, Hemodialysis and Transplantation. Contributions to Nephrology. Vol. 176. Basel, Switzerland: Karger; 2012. pp. 42–53.

27. Ohsawa M, Kato K, Itai K, *et al.* Standardized prevalence ratios for chronic hepatitis C virus infection among adult Japanese hemodialysis patients. Journal of Epidemiology. 2010;20(1):30–39.

28. Kato A, Takita T, Furuhashi M, Fujimoto T, Suzuki H, Maruyama Y. Association of HCV core antigen seropositivity with long-term mortality in patients on regular hemodialysis. Nephron Extra. 2012;2:76–86.

Table 1: The prevalence rate of HCV ribonucleic acid (RNA), and associated odds ratio for different sex and age of a sample of hemodialysis patients in Sana'a city:

Characters	Positive HCV n=17		OR	*CI*	χ^2	*P*
	No	%				
Sex						
Male n=56	8	14.3	1.7	0.6-4.7	1.16	0.27
Female n = 103	9	8.7	0.67	0.21-2.2	0.57	0.45
Age groups						
< 20 Yrs n=13	1	7.7	0.73	0.11-5.2	0.09	0.76

20 - 29 Yrs n=35	2	5.7	0.5	0.1-2.4	0.41	0.33
30 - 39 Yrs n=23	2	8.7	0.83	0.12-4.3	0.06	0.81
40 - 49 Yrs n=22	3	13.6	1.5	0.3-6.4	0.36	0.54
> 49 Yrs n=66	9	13.6	1.6	0.6-4.6	1.02	0.31
n= 159 crude rate	17	10.7				

OR   Odds ratio ≥ 1 is at risk of infection

CI   Confidence intervals----

$\chi 2$ Chi-square ≥ 3.83 is   significant

p   Probability value ≤ 0.05 is significant

Table 2. The prevalence of different HCV genotyping among HCV HD patients, Sana'a city, Yemen

Genotypes	Number	Percentage
Genotype 1a+1b	5	29.4
Genotype 2a	1	5.9
Genotype 4	11	64.7
Genotype 3a	0	0
Genotype 5	0	0
Genotype 6	0	0
Total	17	100

Table 3: The prevalence rate of different HCV markers in 159 haemodialysis patients in Sana'a city

Tests		HCV ribonucleic acid (RNA)		Total
		positive	Negative	
HCV antibodies				
Positive		12	2	14
Negative		5	140	145
HCV core proteins				
Positive		16	0	16
Negative		1	142	143
Total		17	142	159

Table 4: The performance of HCV antibodies test and HCV Core protein test comparing to HCV ribonucleic acid (RNA) among haemodialysis patients in Sana'a city

Evaluated tests	Sensitivity	Specify%	FP %	FN%	PPV%	NPV%
ELISA HCV antibodies	70.6%	98.9%	1.41%	29.4%	85.7%	96.6%
ELISA HCV core protein (core antigen)	94.1%	100%	0.0%	5.9%	100%	97.9%

Sensitivity: Probability that test will be positive, given the patient is disease. Specify: Probability that the test will be negative, given the patient is disease free. FP=false positive : Probability that test will be positive, known the patient is disease free. FN= false negative: Probability that test will be negative, known the patient is diseased. PPV= positive predictive value: Probability that a patient is diseased, given a positive test. NPV= negative predictive value: Probability that a patient disease free, given a negative test.