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RESEARCH ARTICLE

PREVALENCE AND GENOTYPING OF HEPATITIS C VIRUS IN HEMODIALYSIS PATIENTS AND EVALUATION OF HCV-CORE ANTIGEN TEST IN SCREENING PATIENTS FOR DIALYSIS IN SANA'A CITY, YEMEN

Samira H Hanash[1], Hassan A. Al-Shamahy[2*], Mohammed Hussein Saleh Bamshmous[2]
[1]*Medical Microbiology, Faculty of Medicine and Health Sciences, Taiz University, Republic of Yemen.*
[2]*Medical Microbiology and Clinical Immunology, Faculty of Medicine and Health Sciences, Sana'a University, Republic of Yemen.*

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***Address for Correspondence:**
Dr. Hassan A. Al-Shamahy, Faculty of Medicine and Heath Sciences, Sana'a University, P.O. Box 775 Sana'a, Yemen. Phone: +967-770299847, E-mail: *shmahe@yemen.net.ye*

Abstract

Objective: Hepatitis C virus infection is a constant worldwide public health concern. The prevalence of HCV infection is higher in patients on chronic haemodialysis (HD) than in the general population. 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 a better alternative to NAT techniques for the diagnosis of HCV infection during the window period and whether the sensitivity for antibody detection is preserved.
Methods: Current study includes screening of 159 patients on long-term dialysis by HVC antibodies test, PCR HCV-RNA and HCV core antigen test by commercial tests.
Results: 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 comparing with general population; and genotype 4 is predominant.
Conclusion: 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: Genotype, Haemodialysis, Hepatitis C virus, HCV core antigen, HCV antibodies, PCR HCV-RNA, Yemen.

INTRODUCTION

An estimated 143 million people (2%) worldwide are infected with hepatitis C as reported in 2015[1]. In 2013 about 11 million new cases occurred[1]. It occurs most commonly in Africa and Central and East Asia. 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 such as dental workers and public health center workers, it was 5.5% and 11.5% respectively[5,6]. Limited information is available among patients for dialysis in Yemen. HCV infection is more common among patients for dialysis than in healthy populations worldwide. The Dialysis Outcomes and Practice Patterns Study (DOPPS) reported a 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 diagnosis is usually rooted in the detection of an 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 the haemodialysis units of Al-Thorah hospital. 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 tests were 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 were assessed using PCR. 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). Gender and age groups which are possible associated risk factors for HCV infection were assessed. 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 1 shows 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 prevalence rate of HCV among HD patients was 10.7%, for male patients was 14.3%, higher than 8.7% for female patients. When age was considered, there was an increasing 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.

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.

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; χ^2-Chi-square ≥ 3.83 is significant; *p*-Probability value ≤ 0.05 is significant

Seventeen 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 compared 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%.

Table 2: The prevalence of different HCV genotyping among HCV HD patients.

Genotypes	Number	%
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

DISCUSSION

Information on the prevalence of HCV in the general population and in the various high risk groups such as HD patients, prevalence of genotypes and evolution of tests used for screening HCV are important in the 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 CKD patients[15].

Table 3: The prevalence rate of different HCV markers in 159 haemodialysis patients.

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

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 continent[18] had reported an independent and significant association between positive anti-HCV antibody and mortality risk (adjusted relative risk, 1.17; $p<0.0159$).

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.

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 prevalence rate of HCV among HD patients was 10.7%, (male rate=14.3%, female rate=8.7%). This result is indicative 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 reported an overall prevalence of 13.5% among adult hemodialysis patients randomly selected from 308 dialysis facilities in developed countries[7].

In addition, current 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 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 evaluate the performance of HCV antibodies test and HCV Core protein test comparing to HCV ribonucleic acid (RNA) among 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%. Current results are 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, it is not suitable for routine screening[7]. Thus from current results HCV core antigen quantification assay has 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%). Current 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 current study 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 it a routine assay useful for chronic dialysis treatment patients.

CONCLUSIONS

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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AUTHOR'S CONTRIBUTION

This research work is part of A MSc. thesis. **Bamshmous MHS:** conducted the laboratory and field works; and wrote up the thesis. **Al-Shamahy HA:** supervised the laboratory and field works, revised and edited the thesis draft and the manuscript. **Hanash SH:** revised and edited the thesis and the article. All the authors approved the finished version of the manuscript.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

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