INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

Prevalence of Occult Hepatitis C in Chronic Hemodialysis Patients in Mansoura University Hospital, Egypt.

Nahla Anber[1], Mostafa Abd El Salam[2], Ahmed Mohammed Abd El Wahab[2], and Maysaa El Sayed Zaki[3]

[1]Fellow of Biochemistry, Emergency hospital, [2]Internal Medicine, Mansoura Nephrology and Dialysis Unit, [3]Clinical Pathology, Mansoura Faculty of Medicine, Egypt.

ABSTRACT

Hepatitis C infection is a major threat for patients with end stage renal disease under hemodialysis (CHD). Occult hepatitis C infection (OCI) is a recent terminology describing the presence of HCV-RNA in peripheral blood mononuclear cells (PBMC) without evidence of serological marker or presence of RNA in blood. There are scarce data about this entity in CHD.

In this study, we aimed to investigate for the first time the prevalence of occult HCV infection in PBMC of CHD patients in one Egyptian center. Moreover, we will try to link the condition to risk factors associated with HCV infection in those patients.

This study was conducted on 93 patients attending Mansoura Nephrology and Dialysis Unit at Mansoura Univeristy Hospitals. Blood samples were obtained from each patient and subjected to virological study of HCV by the presence of specific antibodies and HCV-RNA in blood and PBMC by real time PCR.

HCVab was positive among 51.8% of the patients. HCV-RNA in seum in 32.3%. Occult HCV as defined by the isolated presence of HCV-RNA in PBMC in 9.7%. The mean level of HCV-RNA in serum was 4.1 ± 1.1 x 10^6 IU/ml and in HCV RNA in PBMC was 9.1 ± 1.045 x 10^6IU/ml. Less than half of patients with positive HCVab (43.8%) had HCV-RNA in serum. On the other hand HCV-RNA in PBMC was present in 60% of patients without presence of HCV-RNA in serum. By logistic regression factor analysis, both the duration of dialysis and number of transfused blood units correlate significantly with the presence of occult HCV (P=0.022 & P=0.009) respectively.

This study found a moderate prevalence of occult HCV in CHD patients with high level of viremia. The risk of occult HCV increased with the prolonged duration of dialysis and the frequency of blood transfusions. Further longitudinal studies are required to evaluate the pathognomonic role of this finding and to modify the screening modules for CHD in Egypt.

Keywords: Hemodialysis, hepatitis C infection, HCV RNA and blood transfusion

INTRODUCTION

Infection with hepatitis C virus (HCV) is a serious health problem in Egypt associated with serious complications such as liver cirrhosis and hepatocellular carcinoma. The laboratory diagnosis of HCV depends mainly on detection of specific antibodies for HCV (HCVab) and detection of HCV viremia in serum by reverse transcriptase polymerase chain reaction (RT-PCR) [1,2]. In the last few years by use of molecular technology diagnostic tools HCV RNA has been detected in non serum samples like hepatocytes and peripheral blood mononuclear lymphocytes. This phenomenon has been called occult HCV infection[3-4]. Later on the term occult HCV has been expanded to include presence of HCV viremia in ultracentrifuge serum samples without detection of HCVab[5]. In patients with spontaneous/treatment-induced HCV-RNA clearance from serum (anti-HCV-positive, serum HCV-RNA-

negative, normal liver transaminases)[6,7]. The gold standard diagnostic tool for diagnosis of OCI is detecting HCV-RNA in hepatocytes in liver biopsy[8]. The clinical course of OCI appears to be milder than that of HCV infection. However, this infection leads to minimal changes in the liver and there are some reports that OCI is associated with complications squeal like HCV infection[9]. The presence of OCI has been reported in many population categories like in family members of OCI patients, in cryptogenic apparently healthy population[9, 10]. Moreover, OCI has been reported in the patients on hemodialysis (CHD) [11] leading to increase the difficulty of controlling HCV infection in dialysis unit that was thought to be easy [12]. Another difficulty in diagnosis of OCI serum transaminases may be normal in these patients even in the presence of liver disease[13,14], The presence of replicating HCV virus in PBMC is a leading cause of increasing virus infection pools in our community.

Unfortunately several studies have shown non satisfied results for the response of OCI to the old therapeutic regimen with pegylated interferon plus ribavirin might[15,16]. There is no adequate data about the use of the new antiviral therapy in those patients.

The hypothesis of ability in persistence of low HCV-RNA in PBMC in normal population can lead to question about the frequency of this condition on patients with impaired immune functions like CHD[17] especially in high endemic area like Egypt. To our best of knowledge there is no data about the prevalence of OCI in Egyptian hemodialysis patients.

In this study, we aimed to investigate for the first time the prevalence of occult HCV infection in PBMC CHD in one Egyptian center. Moreover, we will try to link the condition to risk factors associated with HCV infection in those patients.

MATERIALS AND METHODS

Study design

This study was conducted on 93 patients attending Mansoura Nephrology and Dialysis Unit at Mansoura Univeristy Hospitals. They were 53 males and 40 females with age range from 26 to 65 years. They were complaining of end stage renal disease requiring regular hemodialysis. The study was started from January 2015 to August 2015. We excluded patients with other causes of liver dysfunction (i.e., primary biliary cirrhosis, autoimmune hepatitis, continued alcohol abuse, autoimmune hepatitis, and HIV infection), and also who were being treated with interferon and/or ribavirin. We obtained complete medical history for each patient, including age, location of residence, HBV vaccination history, blood transfusion history, duration of hemodialysis, etiology of end-stage renal disease.

All patients also underwent a complete physical examination. The study was approved by Mansoura Faculty of medicine ethical committee and approval written consent was received from each subject participated in the study.

Ten millitre of blood samples were obtained from each subject and divided into two tubes, one without anticoagulant for sera separation and one with citrate. Serum sample for each subject was distributed into three aliquots. One for full biochemical tests for liver including alanine aminotransferase (ALT) aspartate aminotransferase (AST), bilirubin, and albumin. The other aliquot was used for serological studies by enzyme linked immunosorbant assay for hepatitis C virus IgG (HCV

IgG (Dia-Pro ANTI-HCV, ITALY). The third sera aliquots were kept frozen at 70° C for further molecular study for hepatitis C virus RNA detection. PBMC were immediately prepared from the citrated blood by the standard density gradient centrifugation on Ficoll-Paque using Leucosep tubes (Greiner Bio One GmbH, Germany), isolated and washed as per the manufacturer's instructions. The cells were then counted using a hemocytometer (Neubauer chamber). Aliquots of approximately 2.5 million cells were stored at -80^{o}C until further analysis.

HCV-RNA detection by Real Time PCR

DNA purification was performed using the King Fisher Blood DNA Kit (Cat. No. 97010196) in combination with both the King Fisher Duo and King Fisher Flex magnetic particle processors. DNA was purified from 150 µl to 1 ml of buffy coat samples, and the reagents were titrated accordingly. The buffy coat layer, containing most of the white blood cells and platelets, is situated between the plasma and erythrocytes. Plasma (~55% of total blood) Buffy Coat (HCV and the purifications were carried out according to manufacturers' instructions. Viral nucleic acids were purified from 200 µL aliquots of infected plasma samples using the King Fisher Pure Viral NA Kit (Cat. No. 98070196 or 98070496) and the King Fisher Flex instrument. One run on the King Fisher Flex or King Fisher Duo lasts approximately 40 minutes. After purification, the viral nucleic acids were eluted into 100 µL of nuclease free water. The volume can, however, be adjusted. Reverse transcription of RNA from HCV samples were performed with Thermo Scientific™ Revert Aid™ Premium Reverse Transcriptase. qPCR was carried out on the Thermo Scientific™ Piko Real™ Real-Time PCR System or on the Applied Biosystems® 7500 Real-Time PCR System with Thermo Scientific™ Maxima™ Probe qPCR Master Mix.

Definition
Occult HCV infection was defined as presence of HCV-RNA in PBMC in the absence of HCV-RNA in serum, irrespective of the anti-HCV status.

RESULTS

The study included 93 patients with end stage renal disease on hemodialysis. They were 53 (57%) males and 40 (43%) females with mean age SD 48.0 ± 10.5. The mean duration of dialysis was 33.5±3.5 months. HCVab was positive among 51.8% of the patients. HCV-RNA in serum in 32.3%. Occult HCV as defined by the isolated presence of HCV-RNA in PBMC in 9.7%. The mean level of HCV-RNA in serum was 4.1 ± 1.1x 10^6IU/ml and in HCV-RNA in PBMC was 9.1 ± 1 0.45 x 10^6IU/ml table 1.
Comparison between the HCVab, HCV-RNA and HCV-RNA in PBMC was summarized in table 2. Less than half of patients with positive HCVab (43.8%) had HCV-RNA in serum. On the other hand HCV-RNA in PBMC was present in 60% of patients without presence of HCV-RNA in serum.
By logistic regression factor analysis, both the duration of dialysis and number of transfused blood units correlate significantly with the presence of occult HCV (P=0.022&P=0.009) respectively, table 3

DISCUSSION

Hepatitis C infection in Egypt is a major health problem with claimed percentage of 20% among healthy population [18]. The prevalence of HCV infection among Egyptian is the highest all over the world with prominent infection with genotype 4 [19].
Among patients, those who have end stage renal disease with regular hemodialysis represented a high risk group for acquiring HCV infection. Screening of those patients is routinely performed by detection of HCVab. In the present study 51.8% of the patients had detectable levels of HCV antibodies. Previous reports stated that HCVab can be detected from 1.9% up to 84.6% in CHD in different geographical regions [9, 10]. Various factors result in susceptibility of those patients to HCV infection, among these factors but not limited to, is potential exposure to infected persons, contaminated equipments and blood transfusions. Some investigators reported that hospital acquired HCV infections were reduced by segregation of infected HCV patients in special units. [20,21]. Other reported that only strict application of hygienic measures reduces the chance for HCV infection[22, 23]. The dialysis unit in our hospital practices segregation of patients with HCV with their machines and this did not appear to reduce the prevalence of HCVab.
Although the isolation of HCV-infected patients was not recommended, the Center of Disease Control and Prevention (CDC) encourages ensuring that appropriate precautions are being properly and consistently used[24]. Detecting HCV infection in patients with end-stage renal disease (ESRD) is crucial in the setting of future renal transplant of these patients, where morbidity and mortality as well as possible viral eradication prior to surgery are important issues[25].
Appropriate detection of HCV infection among CHD can limit the spread of HCV infection by applying strict hygienic control measures. Failure to detect truly viremic patients among CHD can aid in spreading of HCV infection among those with patients with positive HCV IgG without viremia. In the present study less than half of patients with positive HCVab (43.8%) had HCV RNA in serum. The study highlights the importance of confirmation of HCV infection in CHD by mean of molecular method as in non CHD patients. It is important for consideration of future management of those patients if renal transplantation is considered as adequate eradication of HCV should be considered before transplantation to reduce morbidity and mortality[25].
Another important issue in CHD is the presence of occult HCV. Occult HCV infection defined as detection of HCV-RNA in PBMC in absence of HCV-RNA in serum. This condition can led to hospital spread of HCV infection among CHD[14]. The hallmark in spread of HCV infection in those patients is usually its silent course with normal biochemical liver functions and absence of detectable HCVab due to the limitation of the immune system.
Occult HCV was detected among 9 patients (9.7%) in the present study. Similar rate was reported by another study[26] in comparison to the results of Baid-Agrawal et al who found low prevalence of occult HCV infection in German population (0.25% in haemodialysis and 0.5% in kidney transplant)[27]. The rate of occult hepatitis C in CHD varies from 0% up to 45%[28,29].
One surprising finding of the present study is the high level of viremia detected in PBMC 9.1 ± 1.045 x 10^6IU/ml. This could reflect the high endemicity of HCV in Egypt and reflect the consideration of CHD for appropriate antiviral therapy for appropriate control of infection pools to reduce nosocomial transmission of HCV and to consider those patients for future renal transplantation therapy.
The other distinguished finding of the present study is the significant association of both the duration of dialysis and number of transfused blood units with the presence of occult HCV (P=0.022 & P=0.009) respectively.
This finding again supports the hypothesis of hospital acquired HCV infection especially with long duration of dialysis, routine screening of blood donors by

presence of HCVab alone and the presence of occult HCV that spread with limited means of identification. To our best of knowledge, this is the first study that was performed on CHD in one Egyptian centre. HCV infection is known to be endemic in Egypt.

This study finds a moderate prevalence of occult HCV in CHD patients with high level of viremia. The risk of occult HCV increases with the prolonged duration of dialysis and the frequency of blood transfusions. Further longitudinal studies are required to evaluate the pathognomonic role of this finding and to modify the screening modules for CHD in Egypt.

Table 1
Demographic and Laboratory data of patients

Parameter	value
Age	48 ± 10.5
Sex Male Female	 53(57%) 40(43%)
Duration of hemodialysis (Months) Mean± SD	33.5 ± 3.5
ALT (IU/L)	43.5± 3.4
AST(IU/L)	38.4± 2.4
Bilirubin (mg/dl)	0.95± 0.34
Number of blood units	3.5± 2.6
HCVab (+)	48 (51.8%)
HCV-RNA in serum Mean± SD	30 (32.3%) 4.1 ± 1.1 x 10^6IU/ml
HCV-RNA in buffy coat Mean± SD	15 (16.1%) 9.1 ± 1.045 x 10^6IU/ml
Occult HCV (isolated HCV-RNA in PBMC	9(9.7%)

Table 2
Relation between HCVab, HCV RNA in serum and HCV in PBMC

HCV ab& HCV-RNA in PBMC	**HCV- RNA in Serum**		**Total No.(%)**
	Positive No.(%)	**Negative NO.(%)**	
HCVab Positive	21(43.8%)	27(56.2%)	48(100%)
HCV-RNA in PBMC	6 (40%)	9 (60%)	15(100%)

Table 3
Risk factors analysis associated with HCV-RNA in PBMC

Paramter	P
ALT	0.912
Blood units	0.022
Duration	0.009

REFERENCES

1. Moyer VA. Screening for hepatitis C virus infection in adults: u.s. preventive services task force recommendation statement. J. Ann Intern Med, 2013; 159(5): 349–357.
2. Jou JH, Muir AJ. In the clinic. Hepatitis C. J. Ann Intern Med, 2012; 157(11): ITC6-1–ITC6-16.
3. Castillo I, Pardo M, Bartolome J, Ortiz MN, Rodriguez IE, de Lucas S, Salas C, Jiménez JA, Pérez MA, Graus J, López JM, Carreño V. Occult hepatitis C virus infection in patients in whom the etiology of persistently abnormal results of liver-function tests is unknown. J. Infect Dis, 2004; 189(1): 7–14.
4. Castillo I, Rodriguez IE, Lopez JM, Pardo M, Bartolome J, Carreno V. Hepatitis C virus replicates in the liver of patients who have a sustained response to antiviral treatment. J. Clin Infect Dis, 2006; 43(10): 1277–1283.
5. Rezaee MS, Einollahi B. Treatment of occult hepatitis C virus infection: does it need special attention? J. Hepat Mon, 2014; 14(7): e16665
6. Welker MW, Zeuzem S. Occult hepatitis C: how convincing are the current data? J. Hepatology, 2009; 49(2): 665–675.
7. Pham TN, Coffin CS, Michalak TI. Occult hepatitis C virus infection: what does it mean? Liver Int, 2010; 30(4): 502–511.
8. Bokharaei SF, Keyvani H, Monavari SH, Alavian SM, Madjd Z, Toosi MN, Mohammad AH. Occult hepatitis C virus infection in Iranian patients with cryptogenic liver disease. J. Med Virol, 2011; 83(6): 989–95.
9. Carreno V, Bartolome J, Castillo I, Quiroga JA. New perspectives in occult hepatitis C virus infection. J. World J Gastroenterol, 2012; 18(23): 2887–94.
10. Barril G, Castillo I, Arenas MD, Espinosa M, Garcia VJ, Garcia FN, González PE, Alcazar JM, Sánchez C, Diez JC, Martinez P, Bartolomé J, Carreño V. Occult hepatitis C virus infection among hemodialysis patients. J. Am Soc Nephrol, 2008; 19(12): 2288–92.
11. Alavian SM. Diabetes, renal failure and hepatitis C infection: The puzzle should be attended more in future. J. Nephro-Urol Mon, 2011; 3(3): 153–154.
12. Baid AS, Pascual M, Moradpour D, Frei U, Tolkoff RN. Hepatitis C virus infection in haemodialysis and kidney transplant patients. J. Rev Med Virol, 2008; 18(2): 97–115.
13. Fabrizi F, Martin P. Occult hepatitis C virus infection in hemodialysis. J. Am Soc Nephrol, 2008; 19(12): 2248–2250.
14. Castillo I, Bartolome J, Quiroga JA, Barril G, Carreno V. Long-term virological follow up of patients with occult hepatitis C virus infection. J. Liver Int, 2011; **31**(10): 1519–24.
15. Pardo M, Lopez JM, Castillo I, Rodriguez IE, Perez MA, Carreno V. Effect of anti-viral therapy for occult hepatitis C virus infection. J. Aliment Pharmacol The, 2006; **23**(8): 1153–9.
16. Hauser AB, Stinghen AEM, Kato S, Bucharles S, Aita C, Yuzawa Y, Pecoits-Filho R. Characteristics and causes of immune dysfunction related to uremia and dialysis. J. Perit Dial Int, 2008; 28: S183–S187.
17. Arthur RR, Hassan NF, Abdallah MY, el-Sharkawy MS, Saad MD, Hackbart BG, Imam IZ. Hepatitis C antibody prevalence in blood donors in different governorates in Egypt. J. Trans R Soc Trop Med Hyg, 1997; **91(3)**: 271–274.
18. Doss W, Shiha G, Hassany M, Soliman R, Fouad R, Khairy M, Samir W, Hammad R, Kersey K, Jiang D, Doehle B, Knox SJ, Massetto B, McHutchison JG, Esmat G. Sofosbuvir Plus Ribavirin for Treating Egyptian Patients With Hepatitis C Genotype 4. J. Hepatol, 2015; 63(3): 581 5.
19. Harmankaya O, Cetin B, Obek A, Seber E. Low prevalence of hepatitis C virus infection in hemodialysis units: Effect of isolation? J. Ren Fail, 2002; 24(5): 639–644.
20. Taskapan H, Oymak O, Dogukan A, Utas C. Patient to patient transmission of hepatitis C virus in hemodialysis units. J. Clin Nephrol, 2001; 55(6): 477–481.
21. Kalantar-Zadeh K, Miller LG, Daar ES. Diagnostic discordance for hepatitis C virus infection in hemodialysis patients. J. Am J Kidney Dis, 2005; 46(2): 290–300.
22. Jadoul M, Cornu C, van Ypersele de Strihou C. Universal precautions prevent hepatitis C virus transmission: A 54 month follow-up of the Belgian Multicenter Study. The Universitaires Cliniques St-Luc (UCL) Collaborative Group. J. Kidney Int, 1998**;** 53(4): 1022–1025.
23. Centers for Disease Control and Prevention. Recommendations for preventing transmission of infections among chronic hemodialysis patients. MMWR Recomm Rep, 2001; **50**: 1–43.
24. Fabrizi F, Bunnapradist S, Lunghi G, Aucella F, Martin P. Epidemiology and clinical significance of hepatotropic infections in dialysis patients. Recent evidence. J. Minerva Urol Nefrol, 2004; 56(3): 249–257.

25. Oesterreicher C, Hammer J, Koch U, Pfeffel F, Sunder PG, Petermann D, Müller D. HBV and HCV genome in peripheral blood mononuclear cells in patients undergoing chronic hemodialysis. J. Kidney Int, 1995; 48(6): 1967–1971.
26. Rinonce HT, Yano Y, Utsumi T, Heriyanto DS, Anggorowati N, Widasari DI, Lusida MI, Soetjipto, Prasanto H, Hotta H, Hayashi Y. Hepatitis B and C virus infection among hemodialysis patients in yogyakarta, Indonesia: Prevalence and molecular evidence for nosocomial transmission. J. Med Virol, 2013; 85(8):1348–61.
27. Baid AS, Schindler R, Reinke P, Staedtler A, Rimpler S, Malik B, Frei U, Berg T. Prevalence of occult hepatitis C infection in chronic hemodialysis and kidney transplant patients. J. Hepatol, 2014; 60(5): 928–933.
28. Thongsawat S, Maneekarn N, Kuniholm MH, Pantip C, Thungsuputi A, Lumlertkul D, Bannachak D, Nelson KE. Occult hepatitis C virus infection during an outbreak in ahemodialysis unit in Thailand. J. Med Virol, 2008; 80(5): 808–815.
29. Barril G, Castillo I, Arenas MD, Espinosa M, Garcia VJ, Garcia FN, González PE, Alcazar JM, Sánchez C, Diez JC, Martinez P, Bartolomé J, Carreño V. Occult hepatitis C virus infection among hemodialysis patients. J. Am Soc Nephrol, 2008; 19(12): 2288–2292.