The Egyptian Journal of Hospital Medicine (Apr. 2017) Vol.67 (2), Page 547- 552

Hepatitis C Virus in Peripheral Mononuclear Cells in Patients on Regular Hemodialysis

Emad Allam Mohamed, Mostafa Abd Elfattah Elballat, Mostafa Mostafa Kamel El-Awdy and Ahmed Abo-Elyazid Ali
Internal Medicine Department, Faculty of Medicine ,Al-Azhar University

ABSTRACT

Background: Occult hepatitis C virus infection (OCI) was identified as Hepatitis C virus (HCV), characterized by undetectable HCV antibodies and HCV RNA in serum, while HCV RNA is detectable in liver and peripheral blood mononuclear cells (PBMCs) only. Nosocomial transmission in dialysis units maintains a higher prevalence of hepatitis C virus (HCV) infection in patients on maintenance dialysis than in the general population. HCV infection has a detrimental effect on survival in patients on maintenance dialysis and after renal transplantation. The excess risk for death in HCV-positive patients was partially attributed to chronic liver disease with its attendant complications, particularly hepatocellular carcinoma and liver cirrhosis[(1)].

The aim of this study: was to evaluate the hidden infection of hepatitis C virus among regular hemodialysis patients in Bab Al Sharia University Hospital with negative ELISA and PCR by using PCR in mononuclear cells as a marker in the serum of these patients

Patients and methods:in this prospective study, 60 patients with end-stage renal disease on regular hemodialysis(for at least 6 months duration)were included. For all patients thorough medical history, clinical examination, kidney function tests, liver function tests, complete blood count, pelvi-abdominal ultrasound, HCVantibodies, hepatitis C viral RNA, quantitative, HbsAg,. HCV PCR done for all patients in serum and mononuclear cells.. Patients with acute or chronic HCV infection as marked by positive hepatitis C antibody,acute or chronic HBV infections marked by hepatitis B surface antigen,other causes of liver dysfunction (e.g., primary biliary cirrhosis, autoimmune hepatitis, HIV infection) and patient on anti HCV treatment.were excluded.

Results: showed detection of HCV-PCR in PBMCs in the absence of HCV-PCR in plasma; was found in three of the 60 patients (3.3%). All patients had negative HIV, HBsAg, HCV Ab and serum HCV PCR.

Conclusion: it could be concluded that testing for HCV-RNA in PBMCs is more reliable than hepatitis serological markers in identifying patients with an OCI when a liver biopsy is not available.

Keywords: Occult hepatitis C virus infection, regular hemodialysis, liver disease, HCV PCR PMNL, ELISA and serum PCR HCV.

INTRODUCTION

Common problem in HD units is the blood transmitted viral infections, particularly infections caused by hepatitis B virus (HBV), hepatitis C virus (HCV) and Human immunodeficiency virus (HIV). Due to the nature of the HD procedure, safety concerns exist for limiting their spread among HD patients and the staff of the unit [(1)].

Egypt has the highest prevalence rate of hepatitis C virus (HCV) in the world, making it the most challenging public health problem facing the country. Studies show that 14.7% of the Egyptian population carry HCV antibodies [(2)] and 9.8% have an active infection [(3)].

Occult HCV infection was first identified in liver of anti-HCV and serum HCV RNA negative patients with abnormal liver function tests and it was also found that viral RNA could be present in the PBMCs of nearly 70% of these patients. Furthermore, it was demonstrated that occult HCV replicates in these cells [(4)].

By detecting HCV RNA in liver biopsies or in PBMCs, other groups in Japan, Italy, Egypt, Colombia, Pakistan and Iran have confirmed the existence of occult HCV infection in patients with elevated liver enzymes and without conventional HCV markers. Occult HCV infection has also been found in hemodialysis patients who were persistently anti-HCV and serum HCV RNA negative but with abnormal values of liver enzymes, in the family setting of patients with occult hepatitis C and even in

Received: 13 / 03 /2017
Accepted: 23 / 03 /2017
DOI : 10.12816/0037805

healthy subjects with normal alanine aminotransferase (ALT) levels and no clinical evidence of liver disease [(5)].

Since HCV was replicating in the liver and PBMCs of patients with occult HCV infection, it was speculated that it should exist as circulating viral particles but at such low levels that the virions could not be detected even using the most sensitive reverse-transcription polymerase chain reaction (RT-PCR) techniques. This hypothesis was tested by concentrating HCV virions by ultracentrifugation of 2 mL of serum from patients with occult HCV prior to HCV RNA detection by RT-PCR [(6)].

T-cell responses are more frequent and stronger compared with chronic hepatitis C patients, contributing to control the extent of the infection and thus prevent HCV RNA detection in serum [(7)].

PATIENTS AND METHODS

This study has been conducted on 60 patients with end-stage renal disease on regular hemodialysis, from hemodialysis unit, Bab Alsharia University Hospital, Al-Azhar University (for at least 6 months duration) .

For all patients thorough medical history, clinical examination, kidney function tests, liver function tests, complete blood count, pelvi-abdominal ultrasound, HCVantibodies, hepatitis C viral RNA, quantitative, HbsAg,. HCV PCR done for all patients in serum and mononuclear cells. Patients with acute or chronic HCV infection as marked by positive hepatitis C antibody,acute or chronic HBV infections marked by hepatitis B surface antigen,other causes of liver dysfunction (e.g., primary biliary cirrhosis, autoimmune hepatitis, HIV infection) and patient on anti HCV treatment were excluded.

Ethics and patient consent:

The study was approved by AL-Azhar University Ethical committee regulations, and patient consent taken from all patients.

Statistical measures

Data analysis will be performed by SPSS. Descriptive analysis as well as student's t-test will be used. A $P< 0.05$ will be considered statistically Significant.

RESULTS

This study showed detection of HCV-PCR in PBMCs in the absence of HCV-PCR in serum. Out of the 60 patients, only three of them (3.3%) were proved to be seropositive. There was statistically high significant differences between patients with negative HCV PCR PMNL and patients with positive HCV PCR PMNL regarding number of blood transfusions ($p < 0.001$) and show no significant differences between patients with negative HCV PCR PMNL and patients with positive HCV PCR PMNL regarding age, sex, duration of hemodialysis and etiology of hemodialysis and transfer between units ($p > 0.05$) as showen in table (1), there were no significant differences between patients with negative HCV PCR PMNL and patients with positive HCV PCR PMNL regarding laboratory results ($p > 0.05$) as showen in table (2).

Table (1): Comparison between patients with negative HCV PCR PMNL and patients with positive HCV PCR PMNL regarding demographic characteristics

		Negative HCV PCR PMNL	**HCV PCR PMNL**	**Independent t-test**	
		No. = 58	**No. = 2**	**t/X²***	**P-value**
Age(years)	Mean ± SD Range	51.97 ± 6.61 37 – 65	48.00 ± 9.90 41 – 55	-0.825	0.413
Sex	Female Male	23 (39.7%) 35 (60.3%)	2 (100.0%) 0 (0.0%)	2.897*	0.089
Duration of hemodialysis yrs (years)	Mean ± SD Range	4.51 ± 2.32 1 – 9	4.00 ± 1.41 3 – 5	-0.306	0.761
Etiology of hemodialysis	Adult polycystic Chronic nephrities DM HTN	2 (3.4%) 11 (19.0%) 11 (19.0%) 34 (58.6%)	0 (0.0%) 1 (50.0%) 0 (0.0%) 1 (50.0%)	1.404*	0.705
Number of blood transfusion	No 1 2 3 4 5	23 (39.7%) 23 (39.7%) 11 (19.0%) 1 (1.7%) 0 (0.0%) 0 (0.0%)	0 (0.0%) 0 (0.0%) 0 (0.0%) 0 (0.0%) 1 (50.0%) 1 (50.0%)	60.000	0.000
Transfer between units	No Yes	55 (94.8%) 3 (5.2%)	2 (100.0%) 0 (0.0%)	0.109	0.741

*: Chi-square test
P-value > 0.05: non significant
P-value < 0.05: significant
P-value < 0.01: highly significant

Table (2): Comparison between patients with negative HCV PCR PMNL and patients with positive HCV PCR PMNL regarding laboratory results

Parameters		Negative HCV PCR PMNL	HCV PCR PMNL	Independent t-test	
		No. = 58	No. = 2	t	P-value
HB (g/dl)	Mean ± SD	10.16 ± 1.50	9.50 ± 0.71	-0.619	0.539
TLC (10^3 cells/ cubic millimeter of blood).	Mean ± SD	6.80 ± 2.14	8.50 ± 0.71	1.115	0.269
Platelet (10^3 cells/ cubic millimeter of blood).	Mean ± SD	233.36 ± 72.66	218.00 ± 53.74	-0.295	0.769
Albumin(g/dl)	Mean ± SD	3.86 ± 0.35	3.95 ± 0.07	0.386	0.701
INR	Mean ± SD	1.08 ± 0.08	1.17 ± 0.10	1.476	0.145
Alanine Aminotransferase (ALT) **IU/L**	Mean ± SD	22.53 ± 6.81	20.50 ± 3.54	-0.417	0.678
Aspartate Aminotransferase (AST) **IU/L**	Mean ± SD	23.63 ± 5.48	24.00 ± 7.07	0.093	0.926
Total bilirubin (mg/dl)	Mean ± SD	0.85 ± 0.18	0.68 ± 0.15	-1.232	0.223
Direct bilirubin (mg/dl)	Mean ± SD	0.20 ± 0.07	0.05 ± 0.02	-0.877	0.384
Urea (mg/dl)	Mean ± SD	98.95 ± 30.64	143.00 ± 40.81	1.951	0.056
Creatinine(mg/dl)	Mean ± SD	8.62 ± 2.51	7.50 ± 0.71	-0.626	0.534

DISCUSSION

Hepatitis C virus (HCV) infection is a growing global health problem. Its threat as a serious public health problem and disease burden has increased. There is an increase in seroprevalence of HCV infection over the last 15 years reaching 2.8% of the world's population, equating to >185 million global infections [(8)].

Egypt has the highest HCV prevalence rate in the world, estimated nationally at 14.7% of the adult population [(9)].

Chronic HCV infection is a significant cause of morbidity and mortality in patients who have End Stage Renal Disease; ESRD [(10)], where the prevalence of HCV infection in patients on maintenance dialysis is higher than in the general population [(1)].

Prevalence rates of HCV among patients on hemodialysis are somewhat higher in Egypt and Saudi Arabia [(14)]. The seroprevalence of HCV infection among various populations on hemodialysis from different geographic areas in Egypt ranged from 52.3% to 82.3% [(15, 16.)] Interestingly, there is a high HCV seroconversion rate among patients on hemodialysis who were HCV free. In Egypt, 11% seroconversion rate was found in a hemodialysis unit over only 7 months. Seroconversion may be associated with previous blood transfusion, central venous catheter use, switching between dialysis places, improper implementation of isolation procedures and infection control measures. These high seroprevalence and seroconversion

rates raise the possibility of occult HCV infection (OCI) in Egypt [14].

OCI is characterized by detection of HCV-RNA in the liver tissue alone and/or peripheral blood mononuclear cells (PBMCs) with undetectable HCV-RNA or antibodies in the serum [19]. The detection of the antigenomic HCV-RNA (negative-strand HCV RNA molecule) can be assumed to be an indication of active viral replication. Both the genomic and the antigenomic HCV-RNA strands were detected in PBMCs of patients with OCI [4, 19], supporting the hypothesis that HCV is able to replicate in these cells. The proven ability of HCV replication in PBMCs raises the question of potential transmission of infection to other cell types (i.e., liver), or to other individuals through blood transfusions or hemodialysis. In fact, the transmission of infection between OCI carriers and people undergoing hemodialysis are feasible [20, 21].

It was hypothesized that if HCV RNA does persist at low levels in PBMCs, then it should be more easily detectable in patients with impaired immune function, such as those on chronic hemodialysis (CHD) and kidney transplant (KTx) recipients than in immunocompetent patients[22].

In our study, the 60 subjects had an average age ± SD of 51.83 ± 6.66 years and included 35 males (58.3%) and 25 females (41.7%). The subjects had an average dialysis duration of 4.49 ± 2.29 years. Hypertension was the most common disease at 58.3, and it was followed by chronic nephritis (20%), diabetes (18.3%) and adult polycystic (3.3%).

In the study done by **El-Shishtawy *et al*** .[23] , hypertension was the most common disease at 49.1%, and it was followed by diabetes (9.4%), and obstructive uropathy (7.5%). Unknown causes of kidney disease were 11.3%.

We speculated that OCI could still represents an important source of nosocomial HCV transmission among hemodialysis patients in Egypt given the frequency and duration of dialysis and the unique high prevalence of anti-HCV among Egyptian hemodialysis patients. We also thought that patient isolation is important in the case of Egypt; of course parallel to the hygienic precautions. We showed that 23 patients (38.3%) had a history of blood transfusion. All patients were free from schistosomiasis and human immunodeficiency virus.

We found a 3.3% prevalence of OCI among 60 hemodialysis patients at Bab Al Sharia University Hospital. by detecting HCV-PCR in PBMCs of those who tested negative for both anti-HCV and HCV-PCR in their sera.

In this regard, a prevalence of 4.8% OCI was reported among 188 hemodialysis patients in Turkey [24], a rate that is close to ours. In another study, persistence of HCV-RNA in PBMCs was reported in 2 of 11 (18%) hemodialysis patients, in Thailand despite negative HCV-RNA in serum [25] but the number of subjects was too small.

Detection of HCV-PCR in PBMCs in the absence of serological markers of HCV has been almost exclusively reported for patients with liver disease or immunosuppressed patients [20, 26].

Importantly, testing for HCV-PCR in hepatocytes is considered the gold standard for documenting OCI. Because of the invasive nature of liver biopsies and not ethically for research propose , these samples were not obtained and HCV-PCR was investigated in PBMC in this study.

Barril *et al*. [20]have attempted to increase the sensitivity of diagnostic tests for OCI in PBMCs. Interestingly, HCV-RNA in PBMCs identifies between 60% and 70% of the OCI cases suggesting underestimation of OCI.

There are data that strongly suggest nosocomial transmission of HCV infection during hemodialysis. Therefore, the prevention of HCV transmission in dialysis patients is focused on the implementation of hygienic precautions concerning the staff of the hemodialysis units and the sterilization of the dialysis machines. Of major importance is the fact that isolation of HCV-infected patients does not seem to protect against HCV transmission in hemodialysis units and therefore it is not recommended [27].

Our study showed statistically a high significant difference between patients with negative HCV PCR PMNL and patients with positive HCV PCR PMNL regarding number of blood transfusions ($p < 0.001$). **El-Shishtawy *et al*.** [23] found that there was no statistically

significant difference between patients ith positive HCV RNA and negative patients regarding blood transfusion.

REFERENCES

1. **Fabrizi F and Martin P(2008):** Occult hepatitis C virus infection in hemodialysis. J AmSocNephrol.,19:2248–2250.
2. **El-Zanaty F and Way A (2009):** Egypt Demographic and Health Survey 2008. Cairo, Egypt: Ministry of Health, El-Zanaty and Associates, and Macro International.
3. **Esmat, G (2013):** Hepatitis C in the Eastern Mediterranean Region. Eastern Mediterranean Health Journal., 19(7).
4. **Castillo I, Rodriguez-Inigo E, Bartolome J *et al.* (2005):** Hepatitis C virus replicates in peripheral blood mononuclear cells of patients with occult hepatitis C virus infection. Gut, 54: 682–685.
5. **Idrees M, Lal A, Malik FA, et al. (2011):** Occult hepatitis C virus infection and associated predictive factors: the Pakistan experience. Infect Genet Evol., 11: 442-445.
6. **Bartolomé J, López-Alcorocho JM, Castillo I *et al.* (2007):** Ultracentrifugation of serum samples allows detection of hepatitis C virus RNA in patients with occult hepatitis C. J Virol., 81: 7710-7715.
7. **Quiroga JA, Castillo I, Pardo M *et al.* (2006):** Combined hepatitis C virus (HCV) antigen-antibody detection assay does not improve diagnosis for seronegative individuals with occult HCV infection. J Clin Microbiol., 44: 4559-4560.
8. **Mohd Hanafiah K, Groeger J, Flaxman AD *et al.* (2013):** Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. Hepatology, 57: 1333–1342.
9. **El-Zanaty F, Way A (2008):** Egypt Demographic and Health Survey. Ministry of Health, El-Zanaty and Associates, and Macro International.
10. **Guerra J, Garenne M, Mohamed MK *et al.* (2012):** HCV burden of infection in Egypt: Results from a nationwide survey. J Viral Hepat., 19: 560–567.
11. **Ramamurthy M, Muir AJ (2006):** Treatmentof hepatitis C in special populations. Clin Liver Dis., 10: 851–865.
12. **Fabrizi F, Takkouche B, Lunghi G *et al.* (2007):** The impact of hepatitis C virus infection on survival in dialysis patients: Meta-analysis of observational studies. J Viral Hepat., 14: 697–703.
13. **Kosaraju K, Faujdar SS, Singh A *et al.* (2013):** Hepatitis viruses in heamodialysis patients: An added insult to injury? Hepat Res Treat., 8605-14.
14. **Khodir SA, Alghateb M, Okasha KM *et al.* (2012):** Prevalence of HCV infections among hemodialysis patients in Al Gharbiyah Governorate, Egypt. Arab J Nephrol Transplant., 5: 145–147.
15. **Hassan AA, Khalil R (2000):** Hepatitis C in dialysis patients in egypt: Relationship to dialysis duration, blood transfusion, and liver disease. Saudi J Kidney Dis Transpl., 11:72–73.
16. **Soliman T, Gibson A and Glenberg A M (2013)** Sensory motor mechanisms unify psychology: the embodiment of culture. Front. Psychol., 4:885. doi: 10.3389/ fpsyg. 2013.00885
17. **Carreno V, Bartolome J, Castillo I *et al.* (2012):** New prespective in occult hepatitis C virus infection. World J Gastroenterol., 18(23): 2887–94.
18. **Castillo I, Rodriguez-Inigo E, Bartolome J, et al. (2005):** Hepatitis C virus replicates in peripheral blood mononuclear cells of patients with occult hepatitis C virus infection. Gut, 54: 682–685.
19. **Pardo M, López-Alcorocho JM, Castillo I *et al.* (2006):** Effect of anti-viral therapy for occult hepatitis C virus infection. Aliment Pharmacol Ther., 23: 1153-1159.
20. **Barril G, Castillo I, Arenas MD et al. (2008):** Occult hepatitis C virus infection among hemodialysis patients. J Am Soc Nephrol., 19:2288–92.
21. **De Marco L, Manzini P, Trevisan M *et al.* (2012):** Prevalence and follow-up of occult HCV infection in an Italian population free of clinically detectable infectious liver disease. PLoS ONE, 7: e43541.
22. **Hauser AB, Stinghen AE, Kato S *et al.* (2008):** Characteristics and causes of immune dysfunction related to uremia and dialysis. Perit Dial Int., 28:S183–7.
23. **El-Shishtawy S, Sherif N, Abdallh E *et al.* (2015):** Occult hepatitis C virus infection in hemodialysis patients: Single center study. Electron Physician., 7(8): 1619-1625.
24. **Yakaryilmaz F, Gurbuz OA, Guliter S *et al.* (2006):** Prevalence of occult hepatitis B and hepatitis C virus infections in Turkish hemodialysis patients. Ren Fail., 28: 729–735.
25. **Thongsawat S, Maneekarn N, Kuniholm MH *et al.* (2008):** Occult hepatitis C virus infection during an outbreak in a hemodialysis unit in Thailand. J Med Virol., 80: 808–15.
26. **Bokharaei-Salim F, Keyvani H, Monavari SH *et al.* (2011):** Occult Hepatitis C Virus Infection in Iranian Patients With Cryptogenic Liver Disease. J Med Virol., 83:989–95.
27. **Kidney Disease:** Improving Global Outcomes (KDIGO) (2008): KDIGO clinical practice guidelines for the prevention, diagnosis, evaluation, and treatment of hepatitis C in chronic kidney disease. Kidney Int., S1–99.