The Egyptian Journal of Hospital Medicine (Oct. 2015) Vol. 61, Page 653- 669

Prevalence and Risk Factors of Overt- and Occult Hepatitis C Virus Infection among Chronic Kidney Disease Patients under Regular Hemodialysis in Egypt

Essam A. El-Moselhy[1*], Ayman Abd El-Aziz[2*], Salwa A. Atlam[1], Raed H. Mnsour[3*], Hesham H. Amin[4*], Tarek H. Kabil[5*],** and **Ayman S. El-Khateeb[1***]**

Departments of Community and Occupational Medicine[1], Internal Medicine[2], Tropical Medicine[3], Clinical Pathology[4], and Medical Microbiology & Immunology[5]

Faculty of Medicine, Al-Azhar*, Tanta**, and El-Menia*** University

Corresponding author: Prof. Dr. Essam A. El-Moselhy, e-mail: dr_elmoselhy@yahoo.com and dr_elmoselhy1@hotmail.com

ABSTRACT

Background: hepatitis C virus (HCV) infection is a major health problem. It is more prevalent among chronic kidney disease (CKD) patients. Occult HCV infection, a new, entity has been described.

Aims: to find out prevalence of occult HCV infection among CKD patients under regular hemodialysis (RHD) and to define epidemiology of HCV infection among them.

Patients and Methods: a sample of 100 CKD patients under RHD was recruited. A questionnaire form was used to collect data. HCV-RNA was tested in serum to detect overt HCV infection patients. HCV-RNA was tested in peripheral blood mononuclear cells of undetected HCV-RNA patients' serum to determine those with occult HCV infection. Rest of the patients was considered HCV free. Biochemical tests were done to all patients.

Results: prevalence of overt and occult HCV infection among CKD patients under RHD was 34.0% and 27.3%, respectively. Liver and renal function tests were significantly higher among the overt and occult HCV patients compared to negative HCV patients. Liver function tests were significantly lower among the occult compared to overt HCV patients. Overt HCV patients had significant risk factors compared to negative HCV patients; age group 40-59 years (OR=9.34) and rural residence (OR=3.14). Also, significant clinical risk factors were the overweight, history of blood transfusion ≥5 times/year, history of nonmedical bloody manipulations, and history of IV drug abuse (OR=3.23, 5.96, 8.28, 7.08, respectively). Occult HCV patients had significant risk factors compared to negative HCV patients; age group ≥60 years and rural residence (OR=6.25 and 6.73, respectively). Significant clinical risk factor was the history of nonmedical bloody manipulations (OR=11.5).

Conclusions and Recommendations: prevalence of overt- and occult HCV infection is high in CKD patients under RHD; this has important clinical and public health implications. There are many significant socio-demographic and clinical risk factors for these infections. A close monitoring of the HD patients and testing them for HCV-RNA in PBMCs yearly to adopted a proper management. Also, more studies on bigger number of patients are required to understand real epidemiology of this health problem.

Keywords: CKD, hemodialysis, overt HCV, occult HCV, prevalence, HCV-RNA, PBMCs, risk factors.

INTRODUCTION

Hepatitis C virus (HCV) is member of the *Flaviviridae* family. Its genome of ~9.5 kb is a RNA positive strand that encodes a large poly-protein of more than 3000 amino acid residues. HCV has great genetic variability, with 6 major genotypes (GTs); HCV GT-1 to 6 has been described, each containing multiple subtypes; >70.[(1)]

HCV is a worldwide infection associated with an increased disease burden due to liver cirrhosis and considerable mortality. It is estimated that ~170 million people (~3.0% of the world's population) are infected with HCV.[(2)] HCV has significant differences in their global distribution and prevalence.[(3)] Adding to the problem of HCV infection is the presence of occult HCV infection.[(4)]

In Egypt, HCV infection is a major health problem. Egypt has the highest prevalence worldwide[(5)] and it is estimated to be 14.7% among general population.[(6)] However, prevalence is higher among hospitalized patients and special clinical populations.[(7)] HCV infection is prevalent among chronic kidney disease (CKD) patients who are under hemodialysis (HD). So, HD patients belong to a high-risk population.[(2)] Also, it has detrimental

Received:22/9/2014
Accepted:29/9/2015
DOI: 10.12816/0018768

effects on disease progression and patient survival times, although HCV-related liver disease is mostly mild.[8-11] The blood of HD patients is much more likely to contain anti-HCV antibodies (Abs) (7.0%-40.0%) than that of the general population.[12] These percents has been lowered in the past few years because of improved prevention measures like using gloves, single-use material, and the isolation of HCV infected people in dialysis units.[13]

Although the mechanism of HCV replication is not fully understood, it is assumed that virus replication involves the synthesis of a negative strand RNA molecule that acts as a template for production of positive strand or genomic HCV-RNA.[14] Thus detection of the HCV-RNA negative strand is indicative of viral replication. The liver is the main site of virus replication.[15] The virus can also replicates at extra-hepatic sites as peripheral blood mononuclear cells (PBMCs).[13,16] So, it has been proposed that PBMCs could be the source of recurrent HCV infection after liver trans-plantation.[17]

As the first definition of an occult HCV infection was based on detecting HCV-RNA in hepatocytes,[18] the presence of HCV-RNA in the liver is the reference method. However, liver biopsy is not readily available and the newly available noninvasive methods for evaluating fibrosis could make it less common.[13] Also, HCV-RNA can be detected in PBMCs of patients with occult HCV infection.[18, 19] So, an alternative for diagnosing an occult HCV infection could be to look for HCV-RNA in PBMCs.[13] Also, ultrasensitive PCR assay can detect HCV-RNA in plasma or serum, although it is undetectable by conventional PCR. HCV-RNA concentrations of 60-160 copy/mL can be detected in the plasma of occult HCV infection patients using ultra-sensitive PCR assay.[20] All the studies that have described occult HCV infection used different methods to increase chance of detecting low concentrations of HCV-RNA.[21] So, HCV-RNA has been detected in the PBMCs of chronically infected patients,[22] in the central nervous system,[23] and other tissues; spleen, pancreas, thyroid[24] and seminal fluid.[25]

Occult HCV infection was first described in patients with hepatic disorders of unknown origin, persistently elevated liver function tests, they were anti-HCV and serum HCV-RNA negative, and all other known causes of liver disease were excluded. Despite absence of conventional HCV markers, very sensitive PCR cleared 57.0% of these patients (100) had HCV-RNA in the liver specimens. Also, it was found that the antigenomic HCV-RNA strand was detected in hepatocytes of 84.0% of those 57 occult HCV infection patients. This indicates an active viral replication. Further, PBMCs from 70.2% (40/57) of these patients were HCV-RNA positive.[18] Also, occult HCV infection has been described in other different clinical setting; in HD patients who were persistently anti-HCV Abs and serum HCV-RNA negative but with abnormal values of liver enzymes,[26] in the family setting of patients with occult hepatitis C, even in healthy subjects with normal alanine aminotransferase (ALT) levels and no clinical evidence of liver disease,[27] and HCV persistence after achievement of a sustained virological response (SVR).[28]

As HCV is replicated in the liver and PBMCs of patients with occult HCV infection, it is 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 real time reverse transcriptase-polymerase chain reaction (rRT-PCR) technique. Also, HCV-RNA could be detected in the ultracentrifuged serum and PBMCs in patients with occult HCV. Further, anti-core HCV tested by a non-commercial enzyme-linked immunosorbent assay (ELISA) is also found in a good proportion of these patients.[26] So, when occult HCV infection is suspected and a liver biopsy is not available, diagnosis can be made, using a highly sensitive rRT-PCR technique, for testing the presence of HCV-RNA in PBMCs, which identifies ~70.0% of the cases[18] or using ultracentrifuged serum, which identifies occult HCV in ~60.0% of the cases.[29,30]

Epidemiological and clinical data about occult HCV infection in CKD patients under HD is scanty.[4,18,31] Also, it is difficult to evaluate the natural history of HCV in HD patients because the exact date of infection is often unknown and it can be silent for several years.[32] In Egypt, to best of our knowledge, the studies on prevalence and risk factors of occult HCV infection is absent or scanty. It is an important public health

problem especially among CKD patients under HD; however this research problem is not fully studied.

OBJECTIVES OF THE STUDY

I- Ultimate objective

To improve health of CKD patients under HD through prevention transmission of HCV in HD units in Egypt between occult HCV infection patients and patients without HCV infection.

II- immediate objectives

1- To find out prevalence of overt- and occult HCV infections among CKD patients under HD.
2- To define the socio-demographic, clinical, and etiological risk factors of these patients.
3- To determine the replication of occult HCV in PBMCs.

PATIENTS AND METHODS

Study setting and design: The present study was conducted at HD unit, El-Ryan Hospital in Cairo, Egypt. This unit was chosen purposively, as one of the researchers (Dr. Ayman Abd El-Aziz) practice there. Most of patients in this HD unit were under umbrella of health insurance. A cross-sectional, analytical study design was chosen to investigate the present research problem.

Administrative and ethical considerations: Approval to conduct this study at the chosen HD unit was obtained. Also, the study protocol was reviewed and got approval from the HD unit's director. A verbal consent was taken from each patient before participating in the study. Aims of the study and procedures that will be taken were cleared for each patient. The researchers assured that patients have the right to withdraw from the study at any time without compromising their rights for clinical care and treatment, investigations will be non-invasive and for the patients' benefit, and data of the patients will be for the purpose of scientific research only and will be handled with confidentially.

Target population and study sample: The CKD patients under regular (R) HD, attending the HD unit, between June 2014 and May 2015, were the target population. A consecutive sample of 100 patients was assayed for HCV-RNA infection in the serum (stage 1) to diagnose cases with HCV infection. Then, the HCV-RNA negative patients were tested for presence of occult HCV infection in their PBMCs (stage 2). So, finally there were three groups of CKD patients; those who have HCV positive at the stages 1 [studied group I (overt/ classic HCV infection)], those who have occult HCV infection (studied group II; detectable HCV-RNA in PBMCs), and those who were HCV-RNA negative patients at stages 1 and 2 [group III=control group (only CKD)].

Inclusion criteria for CKD patients under RHD: Adult patients (age $\geq$ 21 years) and under RHD (4 hour session, 3 sessions/week for at least 1 year).

Exclusion criteria for CKD patients under RHD: Poorly controlled diseases (autoimmune, pulmonary, cardiac, psychiatric, and/or neuro-logical) and co-infection with HBV or HIV.

Tools of the study:

i- Interviewing questionnaire

A specially designed interviewing questionnaire form was used to collect data related to topic of the study.

ii- Clinical examination

The CKD patients' groups were subjected to thorough clinical examinations.

iii- Laboratory tools and methods

Ten ml blood was collected, after the HD secession, from each patient by vacuum venipuncture, using a dry tube. The serum was separated, centrifuged, aliquots and stored at -80°C. Another blood sample for the separation of PBMCs was collected into 10-ml tubes containing heparin as anticoagulant. Immediately after collection, the cells were separated from whole blood by centrifugation on a Ficoll-Hypaque density gradient (density 1076). The pellet of PBMCs was washed for more than three times using phosphate-buffered saline (PH=7.3±0.1). The cells were counted and after adding RNALater (Ambion Inc., Austin, TX) solution, were stored at -80°C until testing.

1- RNA Extraction from Serum and PBMC: RNA was extracted from serum samples by use of the QIAamp Viral RNA Kit (Qiagen). For PBMC samples, the number of cells available was limited. Because the number of cells varied per sample (range, 1.4-7.6 x 10^6 cells/mL), we normalized all quantitative HCV-

RNA data on the PBMC compartment to the copy number of a housekeeping gene, glyceraldehyde-3- phosphate dehydrogenase (GAPDH). Five hundreds µl of a PBMC suspension was washed with diethopyro-carbonate (DEPC)-treated distilled H_2O, and cellular RNA was extracted by use of TRIzol (Invitrogen). The resultant RNA was resuspended in 40 ml of DEPC-treated dH_2O and treated 2 times with DNase I (Ambion).

2- Detection of HCV-RNA Positive Strand: HCV-RNA positive strand was determined by reverse transcription-polymerase chain reaction (RT-PCR). The extracted RNA was subjected for RT-PCR using a one-step RT-PCR kit (QIAGEN, Catalogue no. 210212, sensitivity 22 viral copies) that enables first strand cDNA synthesis and PCR amplification in one reaction mix. Forward (F;5'GCAGAAAGCGTCTAGCC ATGGCGT3') and reverse (R;5'CTCGCAAG CACCCTATCAGGCAGT3') primers (Operon Biotechnologies, Germany) were designed to specifically anneal to conserved regions within the HCV-5'UTR [24] and enable amplification of the 243 bp viral fragment.

The RT-PCR reaction mixture was performed in a final volume of 50 µl in a 0.2 ml nuclease-free Eppendorf tube containing 10 µl RNA template, 10 µl of 5X one-step RT-PCR buffer, 100 pmol of both F & R primers, 2 µl of dNTP's mixture, 2 µl RT-PCR enzyme mix and the volume was completed to 50 µl by nuclease-free water. The PCR tubes were inserted into the heating block of a DNA thermal cycler (Applied Biosystem 271003626, Singapore) and the heating lid was enabled. The RT-PCR was started with first strand, cDNA, synthesis at 50°C for 30 minutes followed by hot start polymerase activation at 95°C for 15 minutes. The PCR amplification program included 36 cycles each consisting of 3 stages for template denaturation at 94°C for 30 seconds, primers annealing at 58°C for 30 seconds, and nucleotides addition (extension) at 72°C for 1 minute. The last cycle was linked to a final extension step at 72°C for 10 minutes followed by cooling at 4°C until the tubes were removed from the machine. The PCR product (174 bp) was submitted to electrophoresis by using a 1.5% agarose gel and was visualized by ethidium bromide staining under ultraviolet light.

3- Detection of the HCV-RNANegative Strand in PBMC: HCV-RNA negative strand was determined by RT-PCR assay according to **El-Awady et al.** [33] Reverse transcription was performed in 25 μL reaction mixture containing 20U of AMV reverse transcriptase (promega, Madison, WI, USA) with 400 ng (3 μL) of total PBMCs RNA, 40U of RNasin (promega, Madison, WI, USA), 0.2 mmol/L from each dNTP (Promega, Madison, WI, USA), and 50 pmol of the forward primer 2CH (for negative strand). The reverse transcription reaction was performed at 42°C for one hour. Amplification of the highly conserved 5' UTR sequences was done using two PCR rounds with two pairs of nested primers. First round amplification was done in 50 μL reaction mixture, containing 50 pmol from each of 2CH (5'AACTA CTGTC TTCACGCAGAA3') forward primer and P2 (5'TGCTCATGGTGCACGGTCTA3') reverse primer, 0.2 mmol/L from each dNTP, 10 μL from RT reaction mixture as template, and 2U of Taq DNA polymerase (Promega, USA) in a 1x buffer supplied with the enzyme.

A positive control RNA of an HCV patient previously tested was included. Moreover, two types of negative controls were included, a negative RT control having no RNA at the reverse transcription step and a PCR negative control having water instead of cDNA. The thermal cycling profile was 1 min at 94°C, 1min at 55°C, and 1 min at 72°C for 30 cycles. The second round amplification was done similar to the first round, except for use of the nested reverse primer D2 (5'ACTCGGCTAG CAGTCTCGCG3') and forward primer F2 (5'GTGCAGCCTCCAGGACCC3') at 50pmol each. PCR products (179 bp) were analyzed on 2% agarose gel electrophoresis.

4- Other virological markers assays: Hepatitis B surface antigen (HBsAg), hepatitis B core antibodies (HBc IgM), and anti HIV Abs.

5- The biochemical investigations: The patient groups were also submitted to the following investigations: **[1]** Renal function tests; blood urea and serum creatinine. **[2]** Liver function tests; ALT, aspartate aminotransferase (AST), total bilirubin, gama glutamyl transpeptidase (GGT), and alkaline phosphatase

(AP). **[3]** Hematological parameters; hemoglobin (Hb), white blood cells count (WBCs), and platelets count.

Statistical analyses:

Statistical analysis included coding, data entering, and sorting by Microsoft office 2010 and statistical analysis program IBM, statistical package for social studies (SPSS) version 20. For quantitative variables, mean (M) ± standard deviation (SD) was calculated. For comparison between means, t-student test was used. For categorical variables, number and percentage were calculated and analytical statistic was done using Chi square (χ^2) test. To determine the risk, odds ratio (OR) was used. Differences were considered statistically significant at P-value <0.05 for $\chi^2$and t-test; while, for OR the 95% confidence interval (CI) or exact confidence limits (ECL) of differences were used to determine the statistical significances.

RESULTS

Sera, of the examined 100 CKD patients under RHD, were assayed for HCV-RNA to find out prevalence of HCV infection among them **(table 1)**. Prevalence of HCV infection among these patients was 34.0% [HCV-RNA positive (overt/classic HCV infection)]. All HCV-RNA negative (66 CKD patients) were tested for presence of HCV-RNA in their PBMCs to find out prevalence of occult HCV infection among them. Prevalence of occult HCV infection among these patients was 27.3% (18/66).Rest of the patients (48) is considered HCV infection free. So, among the entire patients' group there were 34.0% overt HCV infection, 18.0% occult HCV infection, and 48.0% HCV-RNA negative. In addition, HCV-RNA was found by negative strand-specific RT-PCR in PBMCs among 16 out of 18 (88.9%) patients, i.e. had ongoing active HCV replication.

Distribution of CKD patients under RHD with overt and occult HCV infection, and without HCV infection according to their clinical features and mortality rates is shown in table (**2)**. It was obvious that all clinical features (oliguria, generalized edema, disturbed sensorium, associated diseases, and organ transplant) and mortality rates among the three studied groups were statistically insignificant, except presence of jaundice (P=0.004).

Laboratory results of the studied CKD patient with overt-and occult HCV infection, and without HCV infection are illustrated in tables **3**, **4**, and**5**. Means of T. bilirubin, ALT, AST, AP, and GGT in patients with occult HCV infection were lower than that in patients with overt HCV infection with statistically significant differences. While, means of blood urea, serum creatinine, and Hb were lower with insignificant differences **(table 3)**. Meanwhile, all mean laboratory results of patients with occult- and overt HCV infections **(table 4, 5)** were significantly higher than that in CKD patients without HCV infection except Hb was insignificant.

Distribution of CKD patients under RHD with occult- and overt HCV infection according to their socio-demographic, clinical, and etiological risk factors is reported in table **(6)**. As regard the socio-demographic risk factors, the only significant risk factor was the age group ≥ 60 years (OR=5.83, 95% CI: 1.38-26.13). As respect clinical risk factors, the only significant risk factor was the obesity (OR=5.8, 95% ECL: 1.29-27.35). Finally, there were no significant risk factors concerning all etiological factors.

Distribution of CKD patients under RHD with occult HCV and those without HCV infection according to their socio-demographic, clinical, and etiological risk factors is viewed in table **(7)**. Regarding the socio-demographic risk factors, the only significant risk factors were the age group ≥ 60 years (OR=6.25, 95% CI: 1.63-25.06) and the rural residence (OR=6.73, 95% CI: 1.79-26.57). Meanwhile, the only significant protective factors were the age group 21-39 years (OR=0.12, 95% ECL: 0.01-0.59) and urban residence (OR=0.15, 95% CI: 0.04-0.56). Respecting clinical risk factors, the only significant risk factor was the history of nonmedical bloody manipulations (OR=11.5, 95% ECL: 1.69-124.2). Lastly, there were no significant risk factors as respect all etiological factors.

Distribution of CKD patients under RHD with overt HCV and those without HCV infection according to their socio-demographic, clinical, and etiological risk factors is presented in table **(8)**. Concerning the socio-demographic risk factors, the only significant risk factors were

the age group 40-59 years (OR=3.14, 95% CI: 1.14-8.75) and the rural residence (OR=9.34, 95% CI: 3.04-29.79). While, the only significant protective factors were the age group 21-39 years (OR=0.28, 95% CI: 0.09-0.83) and the urban residence (OR=0.11, 95% CI: 0.03-0.33). As regard the clinical risk factors; overweight (OR=3.23, 95% CI: 1.18-9.0), repeated blood transfusion (≥5 times/year) (OR=5.96, 95% ECL: 1.01-61.5), nonmedical bloody manipulations (OR=8.28, 95% ECL: 1.51-82.42), peritoneal dialysis (OR=8.18, 95% ECL: 1.89-48.45), IV drug abuse (OR=7.08, 95% ECL: 1.25-71.55), > 4 years of RHD (OR=3.67, 95% CI: 1.32-10.33), and absence of patients' evaluation for HCV infection twice/year (OR= 5.26, 95% CI: 1.34-24.7) were significant risk factors. The only significant protective factors were obesity (OR=0.24, 95% ECL: 0.06-0.8) and urban residence (OR=0.11, 95% CI: 0.03-0.33). Lastly, there were no significant risk factors as regard all etiological factors.

DISCUSSION

HCV infection is a serious public health problem associated with increased morbidity and mortality. It can lead to the development of cirrhosis and hepatocellular carcinoma.[34] Egypt has the highest prevalence worldwide among general population.[6] Also, in Egypt, HCV prevalence is higher among hospitalized patients and special clinical populations.[7] HD patients belong to the high-risk population.[10,11]

In this study, we reported prevalence 34.0% for overt HCV-RNA, among CKD patients' serum and prevalence 18.0% for occult HCV-RNA in PBMCs, which equals 27.3% of patients with HCV-RNA negative in serum. Significant advances have been made in the study of HCV infection in patients with CKD.[35] Our figures are expected and accepted as HD procedure per se as well as disturbances in both innate and adaptive immunity makes HD patients susceptible to infections.[36]Prevalence of HCV is higher among Egyptian CKD patients under RHD; 35.0% in Al Gharbiyah Governorate, Egypt[37] up to 100.0% in Cairo City.[38] Our figure (34.0%) comes near to Al Gharbiyah Governorate's figure. Also, prevalence of HCV is higher among Egyptian patients referred for bone marrow studies, 42.0%.[39] The prevalence of HCV in HD units was 13.2% in Iran,[40] 16.9% in Brazil,[41] and 20.2% in Turkey.[42] Further, HCV infection still occurs in developed countries (France) HD units, and requires appropriate management, however prevalence of HCV infection has decreased by 7.7%.[43]

Occult HCV infection, a new entity of HCV infection, has not been investigated in Egyptian CKD patients under RHD. We reported prevalence 27.3% occult HCV. The description of occult HCV infection was followed by several large cohort studies looking for trace amounts of HCV in the plasma, PBMCs and/or liver of various populations.[13] A study on 69 aviremic blood donors found no detectable HCV-RNA in their PBMCs.[44] Recently, it is reported that 30.9% of CKD patients under dialysis had occult HCV infections (HCV-RNA detected in PBMCs and/or in ultracentrifuged serum samples).[45] This figure is less than 45.0% that reported previously.[4] Our figure (27.3%) is lower than these figures. So, it is likely that prevalence of occult HCV infection is decreasing due to more efficient diagnostic protocols and transmission preventive measures.[8,11,46]

The discrepancy in the reported prevalence of occult HCV, between studies [4,31,45,47] and ours, could be attributed to many factors; first, the differences in sensitivity of the methods used for detection of the virus genome (nested PCR vs. quantitative rPCR); second, small sample sizes in some of the studies; and third, quantitative differences in the levels of HCV viremia during course of the disease in different patients' populations. This discrepancy is based on data of repeated examined sera from the same patients for presence of HCV-RNA, and showed inconsistent results with previously negative samples being positive for HCV-RNA and vice versa, which suggests a fluctuating level of viremia in the course of the disease. There are also differences in the prevalence of HCV in the general population, according to geographic location that can influence the prevalence of HCV infection among CKD patients under RHD.[48]

It is suggested to use HCV-RNA detection for diagnosis of HCV carriage in areas or HD units with high HCV prevalence, while

anti-HCV Abs detection is better to be used in areas or HD units with low prevalence. Anti-HCV positive test should always be followed by a HCV-RNA test. The latter should also be applied in case of hyper transaminasemia even if the anti-HCV test is negative. The screening with one or other method should be repeated every 6-12 months.[49] So, diagnosis of HCV infection is based on the detection of serological and molecular markers in the serum and plasma.[13]Among patients with occult HCV, 57.0% had serum HCV-RNA after ultra-centrifugation and 61.0% had HCV-RNA in PBMCs.[50]

HCV-RNA in PBMCs has been demonstrated to be reliable for detecting patients with occult HCV infection when a liver biopsy is not preferred; up to 70.0% of patients with occult HCV infection in liver have been found to have HCV-RNA in PBMCs.[18,27] It is important to repeat test on successive samples of PBMCs as HCV-RNA detection is rarely permanent.[13] Test repetition is vital for those with un-detectable HCV-RNA in PBMCs, as this was not performed, so it remains possible that some of occult HCV infection may have been missed.[51] Also, test repetition leads to improve detection of occult HCV infections; up to 100.0%.[52,53] Thus, our result regarding occult HCV infection could be higher if we repeated the test on successive samples of PBMCs, but to reduce financial costs we done the test once only. Also, repeated testing would have little practical applications for routine evaluation in clinical settings.

Occult infections may affect viral reactivation and disease progression, risk of HCV transmission within dialysis units, and intra-familial spread.[4] Patients with false negative results would not be appropriately counseled while undergoing dialysis.[8,10] A major impact of this work is the identification of a subset of HCV seronegative dialysis patients who evade current diagnostic protocols but must be considered potentially infectious, even lacking HCV-RNA amplification in blood.[8]

Genomic HCV-RNA was found in the PBMCs of 45.0% of a group of serum HCV Ab negative/HCV-RNA negative HD patients with elevated liver enzyme activities.[4] This could have an impact on the management of patients under HD. But this result should be interpreted with caution.[54] While, our result showed negative strand specific RT-PCR in PBMCs was 88.9%, which was higher due to the use of samples previously investigated by HCV-RNA.

HCV-RNA detected in plasma of occult HCV infection patients was infectious.[55] If these data are confirmed, occult HCV infection could facilitate clinical reactivation of HCV infection, especially in patients with damaged immune system. The public health impact and significance for blood and organ donation of such situation could be very serious.[13] However, the infection of PBMCs is surprising because they do not have some of the membrane receptors that are essential for HCV entry into hepatocytes. They have no SR-BI, claudin-1 or occludin receptors. Further, cell-culture producing HCV could not replicate in PBMCs.[56]

HCV particles, free or associated with serum apolipoproteins, interact with multiple cell surface proteins on hepatocytes.[57] However, HCV replicates extra-hepatically in chronically infected patients.[58] All CKD patients under HD with detectable HCV-RNA in PBMCs must have viral RNA in the liver. So, it should be noted that detection of HCV-RNA in PBMCs does not identify all cases with occult HCV infection. Thus some of the HD patients without HCV-RNA in PBMCs could have occult HCV infection in liver. However, liver biopsy is not recommended for HD patients.[18]

Positive strand HCV-RNA was detected in 95.0% of liver biopsies and negative strand HCV-RNA (the replication intermediate) was found in 79.0% of liver biopsies that had positive strand HCV-RNA. Thirteen (65.0%) samples of PBMCs had positive strand HCV-RNA; and 12 of those (92.0%) also had negative strand HCV-RNA, none of them had markers of HCV infection or abnormal liver function test. This suggested that virus replication was taking place in the liver of these patients.[59] Another study detected the HCV-RNA genome in the liver biopsies of all 106 (100.0%) patients, in the PBMCs of 69 (65.0%) patients, and in plasma in 62 (58.5%); none of them had markers of HCV infection.[20] Further, a study detected HCV-RNA genome in the hepatocytes of 27 (87.1%) patients, none of whom had markers of HCV

infection or abnormal liver function test. Both positive and negative strands HCV-RNA were found in the livers of 8 (25.8%) patients, suggesting ongoing virus replication in hepatocytes.[60] On the other hand, HCV-RNA was detected among only 5 (20.0%) patients in PBMCs but not detected in plasma of patients with negative serum for HCV-RNA.[61]

The available data on occult HCV infections are conflicting.[13] Many studies [18,26,59,62-64] were in favor of occult HCV infection, while other studies[43,50,65,66-68] supported the recovery of an HCV infection. Several arguments are in favor of the absence of persistent HCV-RNA. However, HCV is an RNA virus that has no latent stage in its replication cycle and its genome cannot persist as DNA, unlike viruses like HIV, HBV, and herpes viruses. It is therefore unclear how low concentrations of HCV can persist.[13]

Among CKD patients under RHD, all clinical features differences, except jaundice, among the three studied groups were statistically insignificant. These results are accepted and expected as 52.0% of the cases had overt or occult HCV infection. Also, the differences between mortality rates indifferent groups were statistically insignificant. Again, the results are accepted and expected as period of the follow up was one year on the maximum, and health conditions and lab results were satisfactory. On the other hand, **Barril*et al.*[4]** showed mortality rates were higher among HD patients with occult HCV infections. However, mortality rates might be changed on the long period of follow up. Further, **Rostaing*et al.*[56]** reported a very high mortality rate (39.0%) but they cleared that these deaths were not due to HCV liver disease. A meta-analysis revealed that in HD patients HCV carriage is associated with 1.57 times increased risk of death. Liver cirrhosis and hepatoma contribute to the increased mortality.[69] Mortality was associated with increasing age, presence of diabetes, coronary artery disease, congestive HF, peripheral vascular disease, and cerebrovascular disease.[70]

As respect laboratory results of the studied patient groups, our results are accepted and expected as HCV affects kidney function and mortality rates are higher among HD patients with occult HCV infections.[4] Also, HCV-infected patients on HD had higher serum ALT levels than those without chronic HCV infection, 44.0±13.5 vs. 33.5±8.0 U/L ($P< 0.001$).[9] Also, levels of ALT and AST were significantly higher in the patients with occult HCV infection compared to patients with negative HCV-RNA in PBMNCs. However, these enzymes were higher in chronic HCV than those of occult HCV infection indicating that the cytolysis is more severe in these cases than in occult HCV infection patients.[71] Further, this difference may be explained by the fact that occult HCV infection patients have a more refined immunological control of HCV infection. So, the breadth of the cellular immune responses is different in the peripheral blood between chronic HCV and occult HCV infection.[72]

ALT and AST levels were in concordance with histological damage of the liver. Necro-inflammatory activity and fibrosis were detected more frequently in chronic HCV than in occult HCV infection patients.[27] So, occult HCV infection seems to be a less aggressive form of the disease caused by overt HCV. However, the existence of occult HCV infection may potentially have significant consequences for this population. These include the risk of nosocomial transmission of the virus within HD units. So, the development of new screening strategies and therapeutic interventions for HCV infection in these patients for detection occult HCV infection may have an important essentiality as it is not discovered by routine lab methods. In addition, data on prevalence of occult HCV are sparse.

The epidemiological studies about occult HCV infection in CKD patients are scanty.[18,31,45] It is difficult to assess the natural history of HCV in these patients as the definite date of contamination is not known in most cases, the infection can be silent for many years, and activities of the liver enzyme cannot be used to predict the development of fibrosis in these patients.[32]

In the present study, the age group ≥60 year represented a significant risk factor for occult HCV group compared to overt HCV patients. A male gender was predominant in all groups of CKD patients, but with insignificant statistical differences. These results come

inconsistent with **Castillo *et al.*[18,26]**, **Natovand Pereira**[35], **Agarwal *et al.*[73]**, and **Saad *et al.*[74]** History of blood transfusion ≥5 times/year was significantly more in patients with overt HCV infection compared to negative HCV-RNA patients, which is in agreement with **Leão*et al.*[9]**, **Natovand Pereira**,[35] **Alavian *et al.*[40]**, and **Agarwal *et al.*[73]** Although screening of blood products for anti-HCV and implementation of precaution measures, HCV infection is still a major problem among CKD under HD.[75] Blood transmitted viral infections, particularly HCV, are important common problems in HD units. Due to the nature of the HD procedure, safety concerns exist for limiting their spread among HD patients and the staff of the unit. Further, the natural history of these infections, the available treatments, and the presence and response to vaccines differ from what is known for the general population.[76] Also, our finding might suggest a role of nosocomial transmission of occult HCV, as reported in HD units for overt HCV infection.[74] However, Belgian multicenter study showed a seroconversion reduction from 1.4% to 0.0% in annual incidence of anti-HCV Ab by full implementation of infection control procedures to prevent transmission of blood borne pathogens, including HCV.[77] Further, we showed history of peritoneal dialysis was found to be a significant risk factor for overt HCV in comparing to negative HCV patients, which is in accordance with **Leão*et al.*[9]**

As respect duration of CKD patients under HD, our result is agree with **Leão*et al.*[9]**, **Natovand Pereira**,[35] **Alavian *et al.*[40]**, and **Agarwal *et al.*[73]** CKD patients under HD presented high susceptibility to acquiring HCV if HD units do not follow the universal precautions recommendations. Accordingly, patients should be evaluated twice/year for HCV and other HCV markers. Patients, who HCV Abs positive are sent to the yellow room (reserved for HCV Abs positive patients), while the seronegative individuals are then tested for anti-HBs Ag.[8] Detection of occult HCV patient in HD units is an essential priority to avoid spread of the virus inside units. The un-identified patients with occult HCV may transmit the infection to other patients who undergo HD alongside them. The repeated exposure to body fluids during dialysis procedures predisposes HD patients to nosocomial transmission of HCV. As HCV-RNA could be detected in the PBMCs of these patients, this indicates active virus replication.

Concerning history of IV drug abuse, it was significantly more in patients with overt HCV infection compared to patients with negative HCV-RNA. Our result is in agreement with **Natovand Pereira**.[35] Also, history of absence of patient evaluation for HCV infection at least twice/year was significantly more in patients with overt HCV infection compared to patients with negative HCV-RNA. Again, our result is in agreement with **Natovand Pereira**.[35] So, strategies to control HCV transmission in HD units should include strict adherence to the universal precautions, careful attention to hygiene, sterilization of dialysis machines and routine serologic testing and surveillance for HCV infection.

CONCLUSIONS AND RECOMMEND-ATIONS

Overt- and occult HCV infection in HD patients is an important clinical and public health problem. In CKD patients under RHD prevalence of overt and occult HCV infection was 34.0% and 27.3%, respectively. Occult HCV infection patients had significantly lower liver function compared to the overt group. Also, there were many significantly demographic (rural residence) and clinical (nonmedical bloody manipulation, history of peritoneal dialysis, etc.) risk factors among HCV patients compared to the controls. We could recommend follow precautions of blood transfusion, a close monitoring of the HD patients and testing them by HCV-RNA in PBMCs once yearly, so that the appropriate management could be adopted, and early renal transplant to reduce exposure to HCV in HD patients. Also, we recommend conducting more detailed studies on bigger numbers and in different areas and populations (e.g., general population, blood donors, etc.) to determine the real epidemiology of this health problem and to fully understanding the virologic, clinical, and public health significance of occult HCV infection in different population groups.

REFERENCES

1. Simmonds P, Bukh J, Combet C *et al.* (2005): Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology, 42(4): 962-73.

2. Yen T, Keeffe EB, and Ahmed A (2003): The epidemiology of hepatitis C virus infection. J ClinGastroenterol., 36: 47-53.

3. Bostan N and Mahmood T (2010): An overview about hepatitis C: A devastating virus. Crit Rev Microbiol., 36: 91-133.

4. Barril G, Castillo I, Arenas MD*et al.* (2008): Occult hepatitis C virus infection among hemodialysis patients. J Am SocNephrol., 19: 2288-92.

5. Frank C, Mohamed MK, Strickland GT*et al.* (2000): The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. Lancet, 355(9207): 887-891.

6. 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.

7. Mohamoud YA, Mumtaz GR, Riome S*et al.* (2013): The epidemiology of hepatitis C virus in Egypt: A systematic review and data synthesis. BMC Infect Dis., 13: 288.

8. KDIGO (Kidney Disease: Improving Global Outcomes) (2008): KDIGO clinical practice guidelines for the prevention, diagnosis, evaluation, and treatment of Hepatitis C in chronic kidney disease. Kidney Int., 73(Suppl 109): S1-S99.

9. Leão JR, Pace FH, and Chebli JM (2010): Infection by hepatitis C virus in patients on hemodialysis: Prevalence and risk fators. Arq Gastroenterol., 47(1): 28-34.

10. Fabrizi F, Dixit V, Messa P *et al.* (2012): Hepatitis C-related liver disease in dialysis patients. Contrib Nephrol., 176: 42-53.

11. Jadoul M and Barril G (2012): Hepatitis C in hemodialysis: Epidemiology and prevention of hepatitis C virus transmission. Contrib Nephrol., 176: 35-41.

12. Rahnavardi M, Hosseini Moghaddam SM, and Alavian SM (2008): Hepatitis C in hemodialysis patients: Current global magnitude, natural history, diagnostic difficulties, and preventive measures. Am J Nephrol., 28(4): 628-40.

13. Nicot F, Kamar N, Rostaing L *et al.* (2011): Occult hepatitis C virus infection: Where are we now? In: Yoshiaki Mizuguchi (Ed.), Liver biopsy in modern medicine, InTech Publisher.

14. Clarke B (1997): Molecular virology of hepatitis C virus. J Gen Virol., 78: 2397-410.

15. Sugano M, Hayashi Y, Yoon S *et al.* (1995): Quantitation of hepatitis C viral RNA in liver and serum samples using competitive polymerase chain reaction. J ClinPathol., 48(9): 820-5.

16. Chang TT, Young KC, Yang YJ *et al.* (1996): Hepatitis C virus RNA in peripheral blood mononuclear cells: Comparing acute and chronic hepatitis C virus infection. Hepatology, 23: 977-81.

17. Laskus T, Radkowski M, Wilkinson J *et al.* (2002): The origin of hepatitis C virus reinfecting transplanted livers: Serum-derived versus peripheral blood mononuclear cell-derived virus. J Infect Dis., 185: 417-21.

18. Castillo I, Pardo M, Bartolomé J *et al.* (2004): Occult hepatitis C virus infection in patients in whom the etiology of persistently abnormal results of liver-function tests is unknown. J Infect Dis., 189: 7-14.

19. Carreño V, Castillo I, Bartolome J *et al.* (2004): Comparison of hepatitis C virus RNA detection in plasma, whole blood and peripheral blood mononuclear cells of patients with occult hepatitis C virus infection. J ClinVirol., 31(4): 312-3.

20. Bartolome J, Lopez-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(14): 7710-5.

21. Pham TN, Coffin CS, and Michalak TI (2010): Occult hepatitis C virus infection: What does it mean? Liver Int., 30(4): 502-11.

22. Blackard JT, Smeaton L, Hiasa Y *et al.* (2005): Detection of hepatitis C virus (HCV) in serum and peripheral-blood mononuclear cells from HCV-monoinfected and HIV/HCVcoinfected persons. J Infect Dis., 192(2): 258-65.

23. Forton DM, Karayiannis P, Mahmud N*et al.*(2004): Identification of unique hepatitis C virus quasispecies in the central nervous system and comparative analysis of internal translational efficiency of brain, liver, and serum variants. J Virol., 78(10): 5170-83.

24. Laskus T, Radkowski M, Wang LF*et al.* (1998): Search for hepatitis C virus extrahepatic replication sites in patients with acquired immunodeficiency syndrome: Specific detection of negative-strand viral RNA in various tissues. Hepatology, 28(5): 1398-401.

25. Bourlet T, Levy R, Maertens A *et al.* (2002): Detection and characterization of hepatitis C virus RNA in seminal plasma and spermatozoon fractions of semen from patients attempting medically assisted conception. J Clin Microbiol., 40(9): 3252-5.

26. Castillo I, Rodríguez-Iñigo E, Bartolomé J *et al.* (2005): Hepatitis C virus replicates in peripheral blood mononuclear cells of patients with occult hepatitis C virus infection. Gut, 54: 682-5.

27. Pardo M, López-Alcorocho JM, Rodríguez-Iñigo E *et al.* (2007): Comparative study between

occult hepatitis C virus infection and chronic hepatitis C. J Viral Hepat., 14: 36-40.

28. Pham TN, Coffin CS, Churchill ND *et al*. (2012): Hepatitis C virus persistence after sustained virological response to antiviral therapy in patients with or without past exposure to hepatitis B virus. J Viral Hepat., 19: 103-11.

29. Carreño V (2006): Occult hepatitis C virus infection: A new form of hepatitis C. World J Gastroenterol., 12: 6922-5.

30. Pankaj J and Sandep N (2008): Occult hepatitis C virus infection is more common than hepatitis B infection in maintenance hemodialysis patients. World J Gastroenterol., 14 (14): 2288-9.

31. Fabrizi F and Martin P (2008): Occult Hepatitis C Virus Infection in Hemodialysis. J Am Soc Nephrol., 19: 2248-50.

32. Furusyo N, Hayashi J, Kanamoto-Tanaka Y *et al*. (2000): Liver damage in hemodialysis patients with hepatitis C virus viremia: A prospective 10-year study. Dig Dis Sci., 45(11): 2221-8.

33. El-Awady MK, Youssef SS, Omran MH *et al*. (2006): Soluble egg antigen of Schistosoma Haematobium induces HCV replication in PBMC from patients with chronic HCV infection. BMC Infect Dis., 6: 91. doi: 10.1186/1471-2334-6-91.

34. Moyer VA (2013): Screening for hepatitis C virus infection in adults: US preventives services task force recommendation statement. Ann Intern Med., 159:349-57.

35. Natov SNand Pereira BJ (2005): Hepatitis C virus in chronic dialysis patients. Minerva Urol Nephrol., 57(3): 175-97.

36. Eleftheriadis T, Kartsios C, Yiannaki E *et al*. (2008): Chronic inflammation and CD16+ natural killer cell zeta-chain down regulation in hemodialysis patients. Blood Purif., 26: 317-21.

37. 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(3): 145-7.

38. El Yazeed SA, Nael G, Younes K *et al*. (2006): Antiphospholipid antibodies in Egyptian patients with chronic renal failure. J Med Sci., 6(3): 468-73.

39. Mousa SM (2014): Hepatitis C among Egyptian patients referred for bone marrow examination: Seroprevalence and analysis of hematological findings. Bone Marrow Res., 2014: 549-52.

40. Alavian SM, Einollahi B, Hajarizadeh B *et al*. (2003): Prevalence of hepatitis C virus infection and related risk factors among Iranian haemodialysis patients. Nephrology, 8(5): 256-60.

41. Santos MAand Souto FJ (2007): Infection by the hepatitis C virus in chronic renal failure patients undergoing hemodialysis in Mato Grosso state, central Brazil: A cohort study. BMC Public Health, 7: 32.

42. 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(8): 729-35.

43. Saune K, Kamar N, Miedouge M *et al*. (2010): Decreased prevalence and incidence of HCV markers in haemodialysis units: A multicentric French survey. Nephrol Dial Transplant., 25: 39-43.

44. Bernardin F, Tobler L, Walsh I *et al*. (2008): Clearance of hepatitis C virus RNA from the peripheral blood mononuclear cells of blood donors who spontaneously or therapeutically control heir plasma viremia. Hepatology, 47(5): 1446-52.

45. Barril G, Quiroga JA, Arenas MD *et al*. (2014): Impact of isolated hepatitis C virus (HCV) core-specific antibody detection and viral RNA amplification among HCV-seronegative dialysis patients at risk for infection.J ClinMicrobiol., 52(8): 3053-6.

46. Kamili S, Drobeniuc J, Araujo AC *et al*. (2012): Laboratory diagnostics for hepatitis C virus infection. Clin Infect Dis., 55(Suppl 1): S43-S8.

47. De Marco L, Gillio-Tos A, FianoV *et al.* (2009): Occult HCV infection: An unexpected finding in a population unselected for hepatic disease. PLoS ONE, 412: e812-8.

48. kazemi-Shirazi L, Petermann D, and Muller C (2000): Hepatitis B virus in sera and liver tissue of HBsAg negative DNA with chronic hepatitis C. J Hepatol., 3: 785-90.

49. Detection and Evaluation of HCV in CKD (2008): Guideline 1. Kidney Int., 73: S10-S9.

50. Castillo I, Bartolomé J, Quiroga JA *et al*. (2010): Diagnosis of occult hepatitis C without the need for a liver biopsy. J Med Virol., 82(9): 1554-9.

51. Baid-Agrawal S, Schindler R, Reinke P *et al*. (2014): Prevalence of occult hepatitis C infection in chronic hemodialysis and kidney transplant patients. J Hepatology, 60(5): 928-33.

52. Pham TN, MacParland SA, Mulrooney PM *et al*. (2004): Hepatitis C virus persistence after spontaneous or treatment-induced resolution of hepatitis C. J Virol., 78(11): 5867-74.

53. Pham TN, King D, Macparland SA *et al*. (2008): Hepatitis C virus replicates in the same immune cell subsets in chronic hepatitis C and occult infection. Gastroenterology, 134(3): 812-6.

54. Kamar N, Nicot F, Izopet J *et al*. (2009): Occult hepatitis C virus infection in hemodialysis patients: Examining the evidence. Am J Kidney Dis., 54(1): 10-2.

55. MacParland SA, Pham TN, Guy CS *et al*. (2009): Hepatitis C virus persisting after clinically apparent sustained virological response to antiviral

therapy retains infectivity in vitro. Hepatology, 49(5): 1431-41.

56. Marukian S, Jones CT, Andrus L *et al*. (2008): Cell culture-produced hepatitis C virus does not infect peripheral blood mononuclear cells. Hepatology, 48(6): 1843-50.

57. Boonstra A, van der Laan LJ, Vanwolleghem T *et al*. (2009): Experimental models for hepatitis C viral infection. Hepatology, 50(5): 1646-55.

58. Durand T, Di Liberto G, Colman H *et al*. (2010): Occult infection of peripheral B cells by hepatitis C variants which have low translational efficiency in cultured hepatocytes. Gut, 59(7): 934-42.

59. Castillo I, Rodríguez-Iñigo E, López-Alcorocho JM *et al*. (2006): Hepatitis C virus replicates in the liver of patients who have a sustained response to antiviral treatment. Clin Infect Dis., 43: 1277-83.

60. Idrees M, Lal A, Malik FA *et al*. (2010): Occult hepatitis C virus infection and associated predictive factors: The Pakistan experience. Infect Genet Evol., 12: 1567-71.

61. Gallegos-Orozco JF, Rakela J, Rosati MJ *et al*. (2008): Persistence of hepatitis C virus in peripheral blood mononuclear cells of sustained viral responders to pegylated interferon and ribavirin therapy. Dig Dis Sci., 53(9): 2564-8.

62. Radkowski M, Gallegos-Orozco JF, Jablonska J *et al*. (2005): Persistence of hepatitis C virus in patients successfully treated for chronic hepatitis C. Hepatology, 41: 106-14.

63. Radkowski M, Horban A, Gallegos-Orozco JF *et al*. (2005): Evidence for viral persistence in patients who test positive for anti-hepatitis C virus antibodies and have normal alanine aminotransferase levels. J Infect Dis., 191: 1730-3.

64. Carreño V, Pardo M, Lopez-Alcorocho JM *et al*. (2006): Detection of hepatitis C virus (HCV) RNA in the liver of healthy, anti HCV antibody-positive, serum HCV RNA-negative patients with normal alanine aminotransferase levels. J Infect Dis., 194(1): 53-60.

65. George SL, Bacon BR, Brunt EM *et al*. (2009): Clinical, virologic, histologic, and biochemical outcomes after successful HCV therapy: A 5-year follow-up of 150 patients. Hepatology, 49(3): 729-38.

66. Wiegand J, Jackel E, Cornberg M *et al*. (2004): Long-term follow-up after successful interferon **therapy** of acute hepatitis C. Hepatology, 40(1): 98-107.

67. Nicot F, Kamar N, Mariame B *et al*. (2010): No evidence of occult hepatitis C virus (HCV) infection in serum of HCV antibody-positive HCV RNA-negative kidney-transplant patients. Transpl Int., 23 (6): 594-601.

68. Swain MG, Lai MY, Shiffman ML *et al*. (2010): A sustained virologic response is durable in patients with chronic hepatitis C treated with peginterferonalfa-2a and ribavirin. Gastroenterology, 139(5): 1593-601.

69. Fabrizi F, Martin P, Dixit V*et al*. (2004): Metaanalysis: Effect of hepatitis C virus infection on mortality in dialysis. Aliment Pharmacol Ther., 20: 1271-7.

70. Goodkin DA, Young EW, Kurokawa K *et al*. (2004): Mortality among hemodialysis patients in Europe, Japan, and the United States: Case-mix effects. Am J Kid Dis., 44(Suppl 2): 16-21.

71. Schneeberger PM, Keur I, van Loon AM *et al*. (2000): The prevalence and incidence of hepatitis C virus infections among dialysis patients in the Netherlands: A nationwide prospective study. J Infect Dis., 182: 1291-9.

72. Quiroga JA, Castillo I, Rodrı´guez-Inigo E *et al*. (2004): HCV-specific cellular immune responses in patients with occult HCV infection in the liver. Hepatology, 40: 278A.

73. Agarwal SK, Dash SC, Gupta S *et al*. (2009): Hepatitis C virus infection in haemodialysis: The 'no-isolation' policy should not be generalized. Nephron Clinic Practice, 111(2): c113-7.

74. Saad Y, Zakaria S, Ramzy I *et al.* (2011): Prevalence of occult hepatitis C in Egyptian patients with non alcoholic fatty liver disease. Open J Intern Med., 1: 33-7.

75. Fabrizi F, Lunghi G, Ganeshan SV *et al*. (2007): Hepatitis C virus infection and the dialysis patient. Semin Dial., 20: 416-22.

76. Eleftheriadis T, Liakopoulos V, Leivaditis K *et al*. (2011): Infections in hemodialysis: A concise review. Part II: Blood transmitted viral infections. Hippokratia, 15 (2): 120-6.

77. Jadoul M, Cornu C, and van Ypersele de Strihou C (1998): 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. Kidney Int., 53: 1022-5.

Table (1): Frequency distribution of chronic kidney disease (CKD) patients under regular hemodialysis (RHD) according to detection of HCV-RNA in their serum and peripheral blood mononuclear cells (PBMCs)

HCV infection among CKD patients under RHD	No. (n= 100)	%
Overt (classic) HCV infection [group I (detectable HCV-RNA by PCR in patients' serum)]	34	34.0
Occult HCV infection [group II (detectable HCV-RNA by PCR in PBMCs only)]		
Among undetectable HCV-RNA in patients' serum [actually (N=66)]	18	27.3
Negative strand-specific RT-PCR in PBMCs (N=18)	16	88.9
HCV infection free patients [group III (undetected HCV-RNA by PCR in serum and PBMCs)		
Among all patients' group [theoretically]	48	48.0
Among undetectable HCV-RNA in patients' serum [actually (N=66)]	48	72.7

Table (2): Distribution of chronic kidney disease (CKD) patients under regular hemodialysis (RHD) With overt and occult HCV infection, and without HCV infection according to their Clinical features and mortality rates

Clinical features and mortality rates	CKD patients' groups (N=100)						χ^2	P-value
	Overt HCV-RNA [group I (N=34)]		Occult HCV-RNA [group II (N=18)]		-Ve HCV-RNA [group III (N=48)]			
	No.	%	No.	%	No.	%		
Oliguria	6	17.7	3	16.7	11	22.9	0.49	0.779#
Generalized edema	4	11.8	2	11.1	9	18.8	1.02	0.599#
Jaundice	9	26.5	3	16.7	1	2.1	10.72	0.004*
Disturbed sensorium	5	14.7	4	22.2	10	20.8	0.634	0.72#
Associated disease(s):								
Cardiovascular (CAD, CHF, etc)	7	20.6	4	22.2	8	16.7	0.347	0.84#
Diabetes mellitus (type I/II)	8	23.5	6	33.3	11	22.9	0.817	0.66#
Others (cerbrovascular diseases)	3	8.8	2	11.1	5	10.4	0.086	0.95#
Organ transplant (renal/liver)	1	2.9	0	0.0	2	4.2	0.782	0.67#
Mortality (during one year)	3	8.8	1	5.6	1	2.1	1.918	0.38#

* = Statistically significant differences. # = Non significant differences.

Table (3): Laboratory results of the studied chronic kidney disease (CKD) patients under regular hemodialysis (RHD) with occult HCV infection and with overt HCV infection

Variables	Chronic kidney disease (CKD) patients' groups		t-value	P-value
	Occult HCV [group II (N=18)]	Overt HCV [group I (N=34)]		
	Mean ± SD	Mean ± SD		
Total bilirubin (mg/dl)	1.51±0.54	1.97±0.68	-2.665	0.005*
ALT (U/L)	63.87±14.15	72.81±18.53	-1.941	0.02*
AST (U/L)	59.44±13.71	71.19±15.36	-2.818	0.003*
AP (U/L)	57.76±14.52	68.43±15.72	-2.449	0.008*
GGT (U/L)	57.16±11.18	72.25±14.82	-4.122	0.000008*
Blood urea (mg/dl)	89.74±28.47	97.32±31.82	-0.876	0.19#
Serum creatinine (mg/dl)	3.16±1.11	3.72±1.18	-1.693	0.05#
Hb (gm/dl)	10.12±2.07	10.32±2.13	-0.328	0.37#

* = Statistically significant differences. # = Non significant differences.

Table (4): Laboratory results of the studied patients' group III [chronic kidney disease (CKD) patients with undetectable HCV-RNA in serum and PBMCs] and group II (CKD patients plus occult HCV infection)

Variables	CKD patients' groups		t-value	P-value
	-Ve HCV [group III (N=48)]	Occult HCV [group II (N=18)]		
	Mean ± SD	Mean ± SD		
Total bilirubin (mg/dl)	0.61±0.20	1.51±0.54	-6.896	0.000*
ALT (U/L)	33.72±8.36	63.87±14.15	-8.501	0.000*
AST (U/L)	29.26±8.37	59.44±13.71	-8.748	0.000*
AP (U/L)	37.34±9.34	57.76±14.52	-5.551	0.000001*
Gama GT (U/L)	51.28±12.62	57.16±11.18	-1.836	0.03*
Blood urea (mg/dl)	72.15±21.91	89.74±28.47	-2.371	0.01*
Serum creatinine (mg/dl)	2.14±0.68	3.16±1.11	-3.65	0.0007*
Hb (gm/dl)	9.71±2.16	10.12±2.07	-0.708	0.241#

* = Statistically significant differences. # = Non significant differences.

Table (5): Laboratory results of the studied patients' group III [chronic kidney disease (CKD) patients with Undetectable HCV-RNA in serum and PBMCs) and group I (CKD plus overt HCV infection)

Variables	CKD patients' groups		t-value	P-value
	-Ve HCV [group III (N=48)]	Overt HCV [group I (N=34)]		
	Mean ± SD	Mean ± SD		
Total bilirubin (mg/dl)	0.61±0.20	1.97±0.68	-11.32	0.000*
ALT (U/L)	33.72±8.36	72.81±18.53	-11.5	0.000*
AST (U/L)	29.26±8.37	71.19±15.36	-14.468	0.000*
AP (U/L)	37.34±9.34	68.43±15.72	-10.314	0.000*
Gama GT (U/L)	51.28±12.62	72.25±14.82	-60.706	0.000*
Blood urea (mg/dl)	72.15±21.91	97.32±31.82	-3.991	0.0001*
Serum creatinine (mg/dl)	2.14±0.68	3.72±1.18	-7.025	0.000*
Hb (gm/dl)	9.71±2.16	10.32±2.13	-1.27	0.103#

* = Statistically significant differences. # = Non significant differences.

Table (6): Distribution of chronic kidney disease (CKD) patients under regular hemodialysis (RHD) with occult HCV and with overt HCV infection according to their socio-demographic, clinical, and etiological risk factors

Variables		CKD patients under RHD				OR (95% CI) OR (95% ECL)*
		With occult HCV infection (n=18)		With overt HCV infection (n=34)		
		No.	%	No.	%	
Socio-demographic risk factors						
Age (year):	21-	2	11.1	8	23.5	0.41 (0.04-2.45)*
	40-	6	33.3	20	58.8	0.35 (0.09-1.34)
	≥ 60	10	55.6	6	17.7	5.83 (1.38-26.13)#[1]
Gender:	Male	10	55.6	23	67.6	0.06 (0.16-2.26)
	Female	8	44.4	11	32.4	1.67 (0.44-6.38)
Marital status:	Single	2	11.1	6	17.7	0.58 (0.05-3.82)*
	Married	13	72.2	18	52.9	2.31 (0.59-10.04)*
	Divorced/widower	3	16.7	10	29.4	0.48 (0.07-2.32)*
Education:	Illiterate, read, and write	13	72.2	23	67.6	1.24 (0.31-5.59)*
	Elementary and secondary	4	22.2	9	26.5	0.79 (0.15-3.55)*
	University and more	1	5.6	2	5.9	0.94 (0.02-19.34)*
Occupation:	Manual works	15	83.3	25	73.5	1.8 (0.36-11.83)*
	Clerical works	2	11.1	7	20.6	0.48 (0.04-3.01)*
	Professional works	1	5.6	2	5.9	0.94 (0.02-19.34)*
Socioeconomic status:	Low	14	77.8	24	70.6	1.46 (0.33-7.54)*
	Middle	3	16.6	8	23.5	0.65 (0.1-3.29)*
	High	1	5.6	2	5.9	0.94 (0.02-19.34)*
Residence:	Urban	6	33.3	9	26.5	1.39 (0.34-5.69)
	Rural	12	66.7	25	73.5	0.72 (0.18-2.96)
Clinical risk factors						
BMI (kg/m^2):	18.5-24.9 (normal weight)	3	16.6	8	23.5	0.65 (0.1-3.29)*
	25-29.9 (overweight)	6	33.3	21	61.8	0.31 (0.08-1.19)
	≥ 30 (obese)	9	50.0	5	14.7	5.8 (1.29-27.35)*#[1]
History of interfamilial HCV infection:	Wife	2	11.1	3	8.8	1.29 (0.1-12.45)*
	Husband	1	5.6	2	5.9	0.94 (0.02-19.34)*
	Others	0	0.0	2	5.9	0.0 (0.0-10.12)*
History of blood transfusion:	Repeated: ≥ 5 times/year	3	16.7	7	20.6	0.77 (0.11-4.06)*
	Occasional: 1-4 time/year	5	27.8	6	17.7	1.79 (0.36-8.51)*
History of bloody manipulation(s) processes:	Medical: Operative:	6	33.3	11	32.4	1.05 (0.26-4.13)
	Surgical	4	22.2	7	20.6	1.1 (0.2-5.26)*
	Dental	2	11.1	4	11.8	0.94 (0.08-7.4)*
	Nonmedical: Tattooing, etc	6	33.3	9	26.5	1.39 (0.34-5.69)
History of peritoneal dialysis		3	16.6	12	35.3	0.37 (0.06-1.73)*
History of intravenous drug abuse		4	22.2	8	23.5	0.93 (0.17-4.27)*
History of IV anti belharzial treatment		8	44.4	7	20.6	3.09 (0.75-13.0)
Duration of RHD (year)	1-4	4	22.2	12	35.3	0.52 (0.1-2.23)*
	> 4	14	77.8	22	64.7	1.91 (0.45-9.66)*
No patients' testing for HCV infection: 2/Y		3	16.6	11	32.4	0.42 (0.07-1.99)*
Etiological risk factors						
Diabetes mellitus		4	22.2	12	35.3	1.93 (0.45-8.39)
Hypertension		2	11.1	8	23.5	1.16 (0.16-6.93)*
Glomerulonephritis		1	5.6	6	17.6	0.94 (0.02-19.34)*
Lupus nephritis		0	0.0	1	2.9	0.0 (0.0-73.67)*

= Significant protective factor #[1] = Significant risk factor

Table (7): Distribution of chronic kidney disease (CKD) patients under regular hemodialysis (RHD) with occult HCV and without HCV infection according to their socio-demographic, clinical, and etiological risk factors

Variables		CKD patients under RHD				OR (95% CI) OR (95% ECL)*
		With occult HCV infection (n=18)		Without HCV infection (n=48)		
		No.	%	No.	%	
Socio-demographic risk factors						
Age (year):	21-	2	11.1	25	52.1	0.12 (0.01-0.59)*#
	40-	6	33.3	15	31.2	1.1 (0.3-4.0)
	≥ 60	10	55.6	8	16.7	6.25 (1.63-25.06)#[1]
Gender:	Male	10	55.6	32	66.7	0.63 (0.18-2.17)
	Female	8	44.4	16	33.3	1.6 (0.46-5.56)
Marital status:	Single	2	11.1	11	22.9	0.42 (0.04-2.3)*
	Married	13	72.2	31	64.6	1.43 (0.39-5.97)*
	Divorced/widower	3	16.7	6	12.5	1.4 (0.2-7.57)*
Education:	Illiterate, read, and write	13	72.2	33	68.75	1.18 (0.32-5.01)*
	Elementary and secondary	4	22.2	9	18.75	1.24 (0.24-5.36)*
	University and more	1	5.6	6	12.50	0.41 (0.01-3.85)*
Occupation:	Manual works	15	83.3	37	77.1	1.49 (0.32-9.41)*
	Clerical works	2	11.1	5	10.4	1.08 (0.09-7.41)*
	Professional works	1	5.6	6	12.5	0.41 (0.01-3.85)*
Socioeconomic status:	Low	14	77.8	35	72.9	1.3 (0.32-6.4)*
	Middle	3	16.6	7	14.6	1.17 (0.17-6.0)*
	High	1	5.6	6	12.5	0.41 (0.01-3.85)*
Residence:	Urban	6	33.3	37	77.1	0.15 (0.04-0.56)#
	Rural	12	66.7	11	22.9	6.73 (1.79-26.57)#[1]
Clinical risk factors						
BMI (kg/m^2):	18.5-24.9 (normal weight)	3	16.6	12	25.0	0.6 (0.1-2.71)*
	25-29.9 (overweight)	6	33.3	16	33.3	1.0 (0.27-3.61)
	≥ 30 (obese)	9	50.0	20	41.7	1.4 (0.41-4.76)
History of interfamilial HCV infection:	Wife	2	11.1	1	2.1	5.0 (0.28-353.75)*
	Husband	1	5.6	2	4.2	1.35 (0.02-27.45)*
	Others	0	0.0	4	8.3	0.0 (0.0-4.07)*
History of blood transfusion:	Repeated: ≥ 5 times/year	3	16.7	2	4.2	4.6 (0.47-58.32)*
	Occasional: 1-4 time/year	5	27.8	7	14.6	2.25 (0.47-9.85)*
History of bloody manipulation(s) processes:	Medical:					
	Operative:	6	33.3	15	31.3	1.1 (0.3-4.0)
	Surgical	4	22.2	9	18.8	1.24 (0.24-5.36)*
	Dental	2	11.1	6	12.5	0.88 (0.08-5.6)*
	Nonmedical: Tattooing, etc	6	33.3	2	4.2	11.5 (1.69-124.2)*#[1]
History of peritoneal dialysis		3	16.6	3	6.3	3.0 (0.36-24.42)*
History of intravenous drug abuse		4	22.2	2	4.2	6.57 (0.81-77.07)*
History of IV anti belharzial treatment		8	44.4	4	8.3	8.8 (1.84-46.33)*
Duration of RHD (year)	1-4	4	22.2	32	66.7	0.69 (0.13-3.05)*
	> 4	14	77.8	16	33.3	1.44 (0.33-7.45)*
No patients' testing for HCV infection: 2/Y		3	16.6	4	8.3	5.2 (0.29-14.51)*
Etiological risk factors						
Diabetes mellitus		4	22.2	6	12.5	2.17 (0.54-8.62)
Hypertension		2	11.1	2	4.2	1.0 (0.15-4.92)*
Glomerulonephritis		1	5.6	0	0.0	0.65 (0.01-7.22)*
Lupus nephritis		0	0.0	2	4.2	0.0 (0.0-14.37)*

= Significant protective factor #[1] = Significant risk factor

Table (8): Distribution of chronic kidney disease (CKD) patients under regular hemodialysis (RHD) with overt HCV and without HCV infection according to their socio-demographic, clinical, and etiological risk factors

Variables		CKD patients under RHD				OR (95% CI) OR (95% ECL)*
		With overt HCV infection (n=34)		Without HCV infection (n=48)		
		No.	%	No.	%	
Socio-demographic risk factors						
Age (year):	21-	8	23.5	25	52.1	0.28 (0.09-0.83)#
	40-	20	58.8	15	31.2	3.14 (1.14-8.75)#[1]
	≥ 60	6	17.7	8	16.7	1.07 (0.29-3.92)
Gender:	Male	23	67.6	32	66.7	1.05 (0.37-2.96)
	Female	11	32.4	16	33.3	0.96 (0.34-2.7)
Marital status:	Single	6	17.7	11	22.9	0.72 (0.19-2.45)*
	Married	18	52.9	31	64.6	0.62 (0.23-1.66)
	Divorced/widower	10	29.4	6	12.5	2.92 (0.83-10.49)
Education:	Illiterate, read, and write	23	67.6	33	68.75	0.95 (0.33-2.71)
	Elementary and secondary	9	26.5	9	18.75	1.56 (0.48-5.05)
	University and more	2	5.9	6	12.50	0.44 (0.04-2.68)*
Occupation:	Manual works	25	73.5	37	77.1	0.83 (0.27-2.57)
	Clerical works	7	20.6	5	10.4	2.23 (0.54-9.78)*
	Professional works	2	5.9	6	12.5	0.44 (0.04-2.68)*
Socioeconomic status:	Low	24	70.6	35	72.9	0.89 (0.3-2.64)
	Middle	8	23.5	7	14.6	1.8 (0.51-6.4)
	High	2	5.9	6	12.5	0.44 (0.04-2.68)*
Residence:	Urban	9	26.5	37	77.1	0.11 (0.03-0.33)#
	Rural	25	73.5	11	22.9	9.34 (3.04-29.79)#[1]
Clinical risk factors						
BMI (kg/m^2):	18.5-24.9 (normal weight)	8	23.5	12	25.0	0.92 (0.29-2.88)
	25-29.9 (overweight)	21	61.8	16	33.3	3.23 (1.18-9.0)#[1]
	≥ 30 (obese)	5	14.7	20	41.7	0.24 (0.06-0.8)*#
History of interfamilial HCV infection:	Wife	3	8.8	1	2.1	4.55 (0.34-243.74)*
	Husband	2	5.9	2	4.2	1.44 (0.1-20.69)*
	Others	2	5.9	4	8.3	0.69 (0.06-5.16)*
History of blood transfusion:	Repeated: ≥ 5 times/year	7	20.6	2	4.2	5.96 (1.01-61.5)*#[1]
	Occasional: 1-4 time/year	6	17.7	7	14.6	1.26 (0.33-4.76)
History of bloody manipulation(s) processes:	Medical:					
	Operative:	11	32.4	15	31.3	1.05 (0.37-2.99)
	Surgical	7	20.6	9	18.8	1.12 (0.33-3.84)
	Dental	4	11.8	6	12.5	0.93 (0.18-4.34)*
	Nonmedical: Tattooing, etc	9	26.5	2	4.2	8.28 (1.51-82.42)*#[1]
History of peritoneal dialysis		12	35.3	3	6.3	8.18 (1.89-48.45)*#[1]
History of intravenous drug abuse		8	23.5	2	4.2	7.08 (1.25-71.55)*#[1]
History of IV anti belharzial treatment		7	20.6	4	8.3	2.85 (0.64-14.39)*
Duration of RHD (year)	1-4	12	35.3	32	66.7	0.27 (0.1-0.76)#
	> 4	22	64.7	16	33.3	3.67 (1.32-10.33)#[1]
No patients' testing for HCV infection: 2/Y		11	32.4	4	8.3	5.26 (1.34-24.7)*#[1]
Etiological risk factors						
Diabetes mellitus		12	35.3	6	12.5	1.12 (0.33-3.84)
Hypertension		8	23.5	2	4.2	0.86 (0.2-3.36)*
Glomerulonephritis		6	17.6	0	0.0	0.69 (0.06-5.16)*
Lupus nephritis		1	2.9	2	4.2	0.7 (0.01-13.97)*

= Significant protective factor #[1] = Significant risk factor