Occult hepatitis C virus infection among Egyptian hemodialysis patients and its potential effect on anemia management

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Background
Hepatitis C virus (HCV) infection is still a main health problem in hemodialysis (HD) patients. The prevalence of occult hepatitis C infection (OCI) in HD patients may be underestimated, and its possible influence on anemia management has not been studied. We aimed to determine the existence of OCI in Egyptian HD patients as well as its possible effect on anemia management.

Patients and methods
This cross-sectional multicenter study included 98 HCV-negative HD patients (negative for both anti-HCV antibody and HCV-RNA), 43 anti-HCV-positive HD patients, and 10 volunteer people matched for age and sex as a healthy control group. Serology test for anti-HCV antibody, reverse-transcription PCR for HCV-RNA (both serum and peripheral mononuclear cell (PMNC)), complete blood count (CBC), liver transaminases, serum iron, serum ferritin, and high-sensitivity C-reactive protein (hsCRP) were done. The average erythropoiesis-stimulating agent (ESA) doses were calculated over 6 months, and ESA resistance index was calculated. The frequency of packed red blood corpuscle (RBC) transfusion for each patient was recorded.

Results
Our HD patients had significant higher levels of serum ferritin (P=0.011), higher serum alanine aminotransferase and aspartate aminotransferase (P=0.002 and 0.006, respectively), higher hsCRP ($P<0.0001$), and significant lower level of hemoglobin ($P<0.0001$) compared with the healthy control group. The prevalence of OCI was 8.16% (8 of 98 patients). OCI patients had significant longer dialysis duration, higher transaminases, higher hsCRP, higher serum ferritin, and higher frequency of packed RBCs transfusion ($P<0.0001$), whereas mean hemoglobin levels and ESA resistance index showed insignificant differences compared with HCV-negative HD patients. Using logistic regression analysis, frequency of packed RBC transfusion and aspartate aminotransferase were the only independent predictors for OCI (P=0.012 and 0.049, respectively), and by multivariate analysis, no significant predictors were found to be associated with anemia in patients with OCI.

Conclusion
The prevalence of OCI in our study was 8.16%. OCI had no effect on anemia managements.

Keywords:
anemia, Egypt, hepatitis C virus, hemodialysis, occult

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Introduction

Hepatitis C virus (HCV) infection is a main problem for the community health worldwide in both developing and developed countries [1]. Despite the screening of blood products for HCV antibody and implementation of universal infection-control precautions, HCV infection is still a main health problem in hemodialysis (HD) patients [2]. Many risk factors have been recognized for HCV infection among HD patients; the number of blood transfusions, dialysis duration [3], and also nosocomial transmissions because of improper infection-control measures are among the most important factors [4]. Occult HCV infection (OCI) is defined as the detection of HCV RNA in the liver or peripheral blood mononuclear cells (PBMCs) of patients whose sera samples tested negative for HCV RNA, with or without the presence of anti-HCV antibodies [5]. It has been suggested that patients with OCI are properly infectious; they have an intact immune response, so they have a milder disease compared with patients with

chronic hepatitis C, and anti-HCV therapy is advisable in this setting [5,6]. Thus, this may have a high influence on the treatment of HD patients in dialysis units [7]. It was decided that the presence of CD8-rich inflammatory infiltrates in the hepatocytes of patients with OCI suggests an ongoing immune response in the liver and confirms the theory of OCI [8]. In chronic kidney disease, Barril *et al*. [2] detected the presence of genomic HCV RNA in PBMCs in 49 of 109 (45%) of serum antibody-negative and RNA-negative HD patients with abnormal liver enzyme levels. Although the most accurate and gold standard method for the diagnosis of OCI is the detection of HCV genome in the liver, testing of HCV-RNA in PBMCs is an alternative procedure when a liver biopsy is not available [2–9]. A major limitation of identifying OCI cases is the lack of standardized universal sensitive method for detecting HCV-RNA in these studies. Thus, the detection rates of HCV-RNA in PBMCs have varied from 0 to 50% [10]. Owing to the existence of OCI, the actual prevalence of HCV infection among HD patients may be underestimated [11]. Anemia is a common problem in HD patients. Owing to the manufacturing of recombinant human erythropoietin in 1989, anemia in HD patients has become successfully managed [12,13]. Patients on HD with HCV infection have higher hemoglobin (Hb) levels compared with HCV-negative patients [14], as the liver may potentially increase the production of erythropoietin secondary to hepatic regeneration following hepatitis infection [15]. To the best of our knowledge, no previous studies has investigated the possible effect of OCI on anemia treatment in HD patients. This study was carried out to determine the existence of OCI in Egyptian HD patients with its possible effect on their anemia management.

Patients and methods

In this cross-sectional multicenter study, we recruited 141 HD patients from three Egyptian HD Centers in Dakahlia government (including HD Units in Mansoura University Hospital). The study was carried out between June and August 2017. The patients were classified as follows: HCV-negative patients (98 patients) who were negative for anti-HCV by enzyme-linked immunosorbent assays and by reverse-transcription PCR (RT-PCR) for serum HCV-RNA and HCV-positive patients (43 patients); they were positive for both anti-HCV antibodies and serum HCV-RNA, and were included as a patient control. Ten volunteer people of matched age and sex were included as a healthy control group. Patients with positive hepatitis B surface antigen and anti-HIV antibodies, decompensated liver failure, active infections, known malignancies, chronic inflammatory disease, hemoglobin (Hb) opathies, and severe lung or cardiac diseases were excluded from the study. A consent was gained from all patients before study enrollment. The study had approval from local ethical committee and Institutional Review Boards (number of registration: R/16.10.65). All participants were subjected to full history and clinical assessment. Liver enzymes [aspartate aminotransferase (ALT) and alanine aminotransferase (AST)] were determined in all patients. The ALT levels above 17 IU/l and the AST levels above 24 IU/l were considered as abnormal [16]. Furthermore, mean monthly complete blood count (CBC) and erythropoiesis-stimulating agent (ESA) dose were recorded. Erythropoiesis-stimulating agent resistance index (ERI) was calculated as weekly weight-adjusted dose of ESAs divided by the Hb level (g/dl). Serum ferritin, serum iron, serum high-sensitivity C-reactive protein (hsCRP), blood pressure, liver enzymes, BMI, and number of packed red blood corpuscle (RBCs) transfusion were recorded retrospectively (6 months back) for all patients. Overall, 2 ml of peripheral blood sample was withdrawn from each participant and collected in a polyethylene tubes containing EDTA as an anticoagulant for DNA isolation, and the extracted DNA was stored at −80°C until the subsequent use for further molecular study for HCV RNA detection. PBMCs were directly organized from the citrated blood by the standard density gradient centrifugation on Ficoll–Paque using Leucosep tubes (Greiner Bio One GmbH, Kremsmünster, Austria), isolated and washed as per the manufacturer's instructions. The cells were then counted using a hemocytometer (Neubauer chamber). Aliquots of ~2.5 million cells were stored at −80°C until further analysis.

Hepatitis C virus RNA detection by real-time PCR

DNA purification was performed using the KingFisher (Thermo Fisher Scientific, Waltham, MA USA) Blood DNA Kit in combination with both the KingFisher Duo and KingFisher 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) and the buffy coat (HCV) purifications were carried out according to manufacturers' instructions. Viral nucleic acids were purified from 200 µl aliquots of infected plasma samples using the KingFisher Pure Viral DNA Kit and the KingFisher Flex instrument. One run on the KingFisher Flex or

KingFisher Duo lasts ~40 min. 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 was performed with Thermo Scientific (Thermo Fisher Scientific, Waltham, MA USA) RevertAid Premium Reverse Transcriptase. QPCR was carried out on the Thermo Scientific PikoReal RT-PCR System or on the Applied Biosystems 7500 RT-PCR System with Thermo Scientific Maxima Probe QPCR Master Mix.

Statistical analysis

Data was presented using SPSS software version 21. Categorical data were analyzed by χ^2-test and stated as numbers and percent. Scale data were introduced as means and SD and analyzed using independent samples *t*-test. Binary logistic regression was done to define the independent predictors for OCI. Differences between groups were considered to be statistically significant when *P* value less than 0.05.

Results

Comparison between patients and healthy controls regarding laboratory parameters

Patients with end-stage renal disease on HD had significant higher levels of ALT, AST, DBP, systolic blood pressure (SBP), hsCRP, and serum ferritin, whereas healthy control group had a significant higher level of mean Hb ($P<0.0001$). However, age, sex, BMI, and mean serum iron showed statistically insignificant differences between both the groups ($P>0.05$). Only eight patients had OCI among HCV-negative HD patients (Table 1).

Comparison between patients' groups (hepatitis C virus-positive and hepatitis C virus-negative groups)

HCV-positive patients had statistically significant higher levels of mean serum ALT, AST, and ferritin, and mean hsCRP; longer dialysis duration; and lower mean ERI and ESA doses compared with HCV-negative patients. However, age, sex, BMI, mean Hb level, mean serum iron, mean SBP, mean DBP, and mean frequency of blood transfusion showed insignificant differences between both groups (Table 2).

Table 2 Clinical and laboratory data of hepatitis C virus-negative and hepatitis C virus-positive patients

Parameters	HCV negative (mean±SD)	HCV positive (mean±SD)	*P* value
Sex (male/female)	61/29	23/20	NS
Age (years)	39.86±10.63	40.79±10.03	NS
Dialysis duration (years)	3.51±1.83	5.57±2.68	**0.007**
BMI (kg/m^2)	27.63±4.63	37.97±5.05	NS
ALT (IU/l)	19.96±7.85	28.16±7.91	**0.000**
AST (IU/l)	24.87±9.37	29.21±7.21	**0.008**
Hb (mean±SD) (g/dl)	10.40±1.45	10.36±1.06	**NS**
hsCRP (mg/dl)	2.56±2.38	4.69±3.36	<**0.0001**
Iron (mean±SD) (mg/dl)	61.51±69.99	59.55±23.51	NS
ESA (mean±SD) (U)	32503.01 ±7677.47	27210.48 ±6435.24	**0.000**
ERI	11.78±3.81	8.23±1.03	**0.000**
Number of BTX (mean±SD)	0.57±0.89	0.39±0.0.63	NS

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BTX, packed RBCs transfusion; ERI, erythropoiesis-stimulating agent resistance index; ESA, erythropoiesis-stimulating agents; Hb, hemoglobin; HCV, hepatitis C virus; hsCRP, high-sensitivity C-reactive protein.

Table 1 Clinical and laboratory data of patient and control group

Parameters	Patient group	Control group	*P* value
Sex (male/female)	89/52	4/6	NS
Age (years)	40.12±10.42	37.20±6.63	NS
Dialysis duration (years)	3.84±2.18	–	–
BMI (kg/m^2)	27.74±4.76	28.93±2.63	NS
SBP (mmHg)	135.82±20.96	122.90±6.06	0.050
DBP (mmHg)	85.42±12.17	77.40±4.62	0.040
ALT (IU/l)	22.46±8.71	13.80±1.69	0.002
AST (IU/l)	26.19±8.97	18.50±3.24	0.006
Hb (mean±SD) (g/dl)	10.39±1.34	12.81±1.18	<0.0001
hsCRP (mg/dl)	3.21±2.69	0.75±0.21	<0.0001
Ferritin (mean±SD) (mg/dl)	347.07±304.66	186.91±41.98	0.011
Iron (mean±SD) (mg/dl)	60.91±59.67	62.40±15.02	NS
ESA (mean±SD) (U)	28843.35±10709.32	–	–
Number of BTX (mean±SD)	0.51±0.82	–	–
ERI	9.08±4.62		
OCI (number of patients)	8	–	–

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BTX, packed RBCs transfusion; DPB, diastolic blood pressure; ERI, erythropoiesis-stimulating agent resistance index; ESA, erythropoiesis-stimulating agents; Hb, hemoglobin; OCI, occult hepatitis C infection; SBP, systolic blood pressure.

Table 3 Clinical and laboratory data of hepatitis C virus-negative patients and patients with occult hepatitis C virus

Parameters	HCV negative (mean±SD)	Occult HCV patients (mean ±SD)	*P* value
Sex (male/female)	61/29	5/3	NS
Age (years)	39.86±10.85	39.87±8.37	NS
Dialysis duration (years)	**3.32±1.74**	5.75±1.16	<0.0001
BMI (kg/m^2)	27.39±4.59	30.41±4.76	NS
SBP (mmHg)	133.72±20.35	140.63±19.72	NS
DBP (mmHg)	84.33±12.13	87.50±11.14	NS
ALT (IU/l)	**18.47±6.27**	36.75±1.28	<0.0001
AST (IU/l)	**23.00±7.12**	45.87±4.70	<0.0001
Hb (mean±SD) (g/dl)	10.38±1.49	10.65±0.89	NS
hsCRP (mg/dl)	2.30±1.76	4.53±2.68	<0.0001
Ferritin (mean ±SD) (mg/dl)	**335.24±107.05**	523.10±183.01	<0.0001
Iron (mean±SD) (mg/dl)	62.30±72.86	52.59±17.33	NS
ESA (mean ±SD) (U)	32778.16 ±7863.99	29407.57±4311.04	NS
ERI	11.95±3.91	9.83±1.63	NS
Number of BTX (mean±SD)	**0.42±0.72**	2.25±0.89	<0.0001

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BTX, packed RBCs transfusion; DPB, diastolic blood pressure; ERI, erythropoiesis-stimulating agent resistance index; ESA, erythropoiesis-stimulating agents; Hb, hemoglobin; HCV, hepatitis C virus; hsCRP, high-sensitivity C-reactive protein; SBP, systolic blood pressure.

Table 4 Clinical and laboratory data of hepatitis C virus-positive patients and patients with occult hepatitis C virus

Parameters	HCV positive patients (mean±SD)	Occult HCV patients (mean±SD)	*P* value
Sex (male/female)	23/20	5/3	NS
Age (years)	40.72±10.09	39.87±8.37	NS
Dialysis duration (years)	4.57±2.69	5.75±1.16	NS
BMI (kg/m^2)	27.96±5.06	30.41±4.76	NS
SBP (mmHg)	139.30±22.27	140.63±19.72	NS
DBP (mmHg)	87.32±11.87	87.50±11.14	NS
ALT (IU/l)	**28.16±7.91**	36.75±1.28	**0.002**
AST (IU/l)	**29.20±7.21**	45.87±4.70	**<0.0001**
Hb (mean±SD) (g/dl)	10.36±1.07	10.65±0.89	NS
hsCRP (mean±SD) (mg/dl)	4.69±3.36	4.53±2.68	NS
Ferritin (mean±SD) (mg/dl)	**634.21 ±464.86**	523.10 ±183.01	**<0.0001**
Iron (mean±SD) (mg/dl)	**59.55±23.51**	52.59±17.33	**NS**
ESA (mean±SD) (U)	**27210.48 ±6435.24**	29407.57 ±4311.04	**NS**
ERI	**8.23±1.03**	9.83±1.63	**NS**
Number of BTX (mean±SD)	0.39±0.62	2.25±0.89	<0.0001

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BTX, packed RBCs transfusion; DPB, diastolic blood pressure; ERI, erythropoiesis-stimulating agent resistance index; ESA, erythropoiesis-stimulating agents; Hb, hemoglobin; HCV, hepatitis C virus; hsCRP, high-sensitivity C-reactive protein; SBP, systolic blood pressure.

Comparison among patients with occult hepatitis C infection and hepatitis C virus-negative and hepatitis C virus-positive patients

Compared with HCV-negative patients, patients with OCI had statistically significant high serum levels of mean serum ALT, AST, hsCRP, and ferritin ($P<0.0001$) and significant higher mean dialysis duration and mean number of packed RBCs transfusion, whereas age, sex, BMI, mean ESA doses, mean ERI, mean SBP, mean DBP, and mean serum iron showed insignificant differences between both groups (Table 3).

On the contrary, significant high levels of mean serum AST and ALT and mean number of packed RBCs transfusion were found in patients with occult HCV versus HCV-positive patients, whereas age, sex, BMI, mean hsCRP, mean ESA, mean ERI, mean SBP, mean DBP, and mean serum iron showed insignificant differences between both groups (Table 4).

Independent predictors of occult hepatitis C virus

By binary logistic regression test using occult HCV, as the dependent variable to analyze the significant predictors for occult HCV, mean serum ALT and AST, mean hsCRP ferritin, mean dialysis duration, and mean number of packed RBCs transfusion were used as covariates. Mean number of packed RBCs transfusion and ALT were the only significant independent predictors for occult HCV in our patients ($P=0.012$ and 0.049, respectively; Table 5).

Predictors for anemia in patients with occult hepatitis C infection

By multivariate analysis, we did not find any significant parameter as a predictor for anemia in patients with OCI (Table 6).

Discussion

HCV infection among HD patients has a wide variable prevalence in different regions worldwide, as well as between centers within the same country, ranging from 5% up to 60% [16]. Egypt has the highest prevalence of HCV infection in the world [17], and up to 50.7% of HD patients are HCV seropositive [18]. Patients with persistence elevation of aminotransferase levels in conjunction with negative HCV antibody and HCV RNA were discovered to have occult HCV infection

Table 5 Binary logistic regression analysis for detection of independent predictors for occult hepatitis C virus in hepatitis C virus-negative hemodialysis patients

Risk variables	*B*	SE	Wald	*d.f.*	Significance	Exp(*B*)	95% CI for exp(*B*)	
							Lower	Upper
Dialysis duration	–0.294	0.523	0.317	1	0.574	0.745	0.267	2.077
Serum ferritin (mg/dl)	0.001	0.005	0.021	1	0.885	1.001	0.991	1.011
Number of BTX (mean±SD)	–2.474	0.979	6.385	1	**0.012**	0.084	0.012	0.574
ALT (IU/l)	–0.281	0.143	3.877	1	**0.049**	0.755	0.571	0.999
AST (IU/l)	0.106	0.112	0.895	1	0.344	1.112	0.892	1.386
Constant	11.009	4.227	6.784	1	0.009	60422.920		

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BTX, packed RBCs transfusion; CI, confidence interval.

Table 6 Multivariate analysis for predictors of anemia in patients with occult hepatitis C infection

Dependent variables	*d.f.*	Mean square	*F*	Observed power	*P* value
Dialysis duration (years)	1	1.500	1.135	0.147	0.330
Serum ferritin (mg/dl)	1	110.125	0.003	0.050	0.959
Serum iron (mg/dl)	1	42.720	0.124	0.060	0.736
Blood TX	1	1.500	2.250	0.245	0.184
ALT (IU/l)	1	1.500	0.900	0.127	0.379
AST (IU/l)	1	70.042	4.954	0.464	0.068

ALT, alanine aminotransferase; AST, aspartate aminotransferase; Blood TX, packed RBCs transfusion.

[19]. Baid-Agrawal *et al.* [20], found that only 0.25% of 407 patients had OCI, whereas in our work, the prevalence of OCI among our patients was 8.16% (8 out of 98). We think the cause of low prevalence of OCI in the former study is owing to the lower limit for diagnosis of positive HCV PCR used by the authors. Moreover, the high prevalence of HCV infection in Egypt can explain the higher OCI prevalence in our patients. Our results are matched with Yakaryilmaz *et al.* [21], who found that the prevalence of OCI among HD patients was 4.8%, but actually after exclusion of patients with positive HCV antibody and patients with dual infection HCV and HBV, the prevalence of OCI became 6.3%. Previous studies also were in line with our finding [22,23]. Patients with OCI had statistically significant higher values of transaminases levels, higher serum ferritin levels, higher hsCRP, longer dialysis duration, and higher frequency of repeated packed RBCs transfusions compared with HCV-negative patients. All these elements are risk factors for OCI, but we found by applying binary logistic regression analysis, repeated packed RBCs transfusions and ALT were the only independent predictors for OCI in our patients. This result may emphasize on the potential risk of HCV transmission by OCI blood product and should raise the awareness of implementing more tight and meticulous supervision for screening all blood donors for HCV antibody and advise highly sensitive PCR for HCV-RNA testing. However, the effect of OCI on hepatocytes and transaminases elevation needs more study to clarify the exact immunopathological mechanism.

In this study, we studied the relation between OCI and anemia management, which is considered as one of the major complications in chronic kidney disease and contributes to the high incidence of mortalities and morbidities especially owing to its harmful cardiovascular effect [22,24]. In this study, mean Hb levels were statistically insignificant between HCV-positive and HCV-negative patients, in disagreement with a previous study [14]. However, there was a statistically significant lower dose of ESA therapy and ERI and higher serum levels of ferritin and hsCRP in HCV-positive patients within the past 6 months when compared with HCV-negative patients. However, Saifan *et al.* [25] found no significant difference in ferritin and mean ESA therapy between both groups, and this is can be explained by the higher Hb level among HCV-infected patient in his study. We also reported that OCI has no effect on Hb level compared with HCV-negative group. On the contrary, we found significantly high ferritin and high hsCRP levels in patients with OCI in comparison with the negative group, which may be attributed to the subclinical inflammatory status [26,27] in such group of patients without statistically significant difference in the ESA dose and ERI between the HCV-negative patients and patients with OCI. In this study, OCI had no effect on anemia management, and no serological or clinical parameters were associated with anemia in patients with OCI by multivariate analysis. However, HCV infection had desirable effect on Hb levels which could be explained by the severity of chronic inflammation in HCV-positive infection,

which may be associated with increased production of endogenous erythropoietin from the regenerating liver cells [28,29] and could be related to lower hepcidin level in patient with HCV infection [30]. Moreover, chronic HCV infection has been associated with oxidative stress activation, which may play a role in the development of local and systemic inflammation. However, in case of OCI, there was a minimal systemic inflammatory state rather than actual chronic hepatitis with regeneration which was supported by our result of higher levels of mean hsCRP in both patients with overt and patients with occult HCV.

As we reported that unexplained high liver transaminases in HD patients can be attributed to OCI which probably carries the risk for nosocomial transmission in such vulnerable patient group. Even though OCI seems to be milder than overt chronic hepatitis C, liver fibrosis exists in up to 5% of the patients [31,32]. Furthermore, necroinflammatory activity is detected in the liver in 35% of the cases [33,34]. This indicates that OCI may progress to a more severe chronic liver injury, so specific anti-HCV therapy is recommended for these patients.

We recommend conducting further prospective studies on a larger number of population. Finally, we conclude that OCI was not uncommon in our patients and repeated packed RBCs transfusion carried high risk for OCI. OCI had no effect on anemia management in our HD patients.

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Conflicts of interest
There are no conflicts of interest.

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