EFFECT OF HEPATITIS C VIRUS INFECTION ON HAEMATOCRIT AND HAEMOGLOBIN LEVELS IN EGYPTIAN HEMODIALYSIS PATIENTS

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Aim: Hepatitis C virus (HCV) infection is common among the Egyptians. This prevalence is higher among hemodialysis (HD) patients in whom anemia is a common finding. Recently, some case reports and few studies indicated that red cell status increased after hepatitis C viral infection among HD patients. The aim of our study is to investigate whether HCV-positive HD patients have higher hemoglobin (Hb) and hematocrit (HCT) values compared to HCV-negative patients.

Methods: Ninety-nine chronic (HD) patients were the subject of this study. Their HCV status was determined by anti-HCV antibodies and confirmed with RNA polymerase chain reaction (PCR). Those with a history of blood transfusion or massive blood loss during the last 6 months were excluded from the study.

Results: 70.7% of our patients tested positive for anti-HCV antibody (56.9 % were male). The mean age for HCV positive group was (40.41±14.17 years) while it was (47.35±19.18 years) for HCV negative group (P=0.08). HCV positive group has a longer hemodialysis duration (66.54 ± 43.92 months) compared to HCV negative patients (30.96±23.17 months, P=0.006). Mean Hb was similar in HCV-positive compared to HCV negative group (10.32±2.03 versus 10.22±1.52 gm/dl respectively) (P=0.63). Mean HCT values were also similar in both groups being 30.94± 6.089% in HCV positive versus 30.77± 4.53% in HCV negative group, respectively (P= 0.094). Fifty-five patients (39 HCV positive and 16 were HCV negative) received erythropoietin (EPO) therapy whilst only twenty patients received IV iron. Mean Erythropoietin dose was 5000±2236.06 Units/week in HCV- positive patients versus 6250±2720.29 Units /week in HCV - negative group (P=0.09). Liver function tests were normal except for alanine aminotransferase (ALT) that was significantly higher among HCV-positive compared to HCV-negative patients (31.75±36.4 vs 15.1±7.21 U/L, P=0.05).

Conclusion: HCV-positive and HCV-negative Egyptian chronic hemodialysis patients have comparable hemoglobin as well as hematocrit levels and the erythropoietin dose was not influential as its lower value in HCV-positive patients did not reach a statistically significant level.

Key words: Hepatitis C virus, Haematocrit, haemoglobin, hemodialysis.

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INTRODUCTION

Approximately, 170 million people worldwide are chronically infected with the hepatitis C virus (HCV) (1). The prevalence of HCV infection in patients undergoing dialysis is persistently greater than that in the general population (2) being endemic in hemodialysis (HD) units around the world, predominantly in Mediterranean and developing countries of the Middle and Far East (3). Nosocomial transmission of HCV infection has been reported to be a considerable route in modern hospital dialysis units, particularly during the outbreaks of infection(4).

Anemia is the most common hematological abnormality in chronic renal failure. In the

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Table 1. Demographic characteristics for the 99 subjects included in the study.

Group/Parameter	HCV positive (70)	HCV negative (29)	p value
Male/female	49/21	19/10	ns
Age (Years , Mean±SD)	40.41±14.17	47.35±19.18	0.08
Original kidney disease			
Unknown	26	13	
Hypertension	11	2	
Diabetes mellitus	5	4	
Graft failure	10	3	
Hereditary nephritis			ns
Glomerulonephritis	1	1	
Systemic lupus erythematosis	2	1	
Pregnancy related	5	1	
Vesicoureteric reflux	5	2	
Stone kidney disease	1	1	
Multiple myeloma	3	1	
	1	0	
Hypertension	26	12	ns
Hemodialysis duration (Months)	66.54 ± 43.92	30.96±23.17	0.006
Dry body weight (Kg)	59.91±14.75	73.78±13.7	0.002
Serum sodium (mg/dl)	138.23±17.54	132.02±23.74	ns
Potassium (mg/dl)	5.15±1.01	4.99±0.83	ns
Alkaline phosphatase (Iu/l)	160.55±194.72	144.33±147.85	ns
Albumin (gm/dl)	3.09±0.52	3.26±0.50	ns
ALT (Iu/l)	31.75±36.4	15.1±7.21	0.05
AST (Iu/l)	27.52±17.02	19.67±7.84	ns
Bilirubin (mg/dl)	0.58±0.24	0.54±0.21	ns
Cholesterol (mg/dl)	173.46±39.59	191.6±41.59	ns
Fasting blood sugar (mg/dl)	108.09±21.11	108.57±28.45	ns
Calcium (mg/dl)	8.97±0.74	9.12±0.94	ns
Phosphorus (mg/dl)	5.63±1.64	5.47±2.24	ns

ns: non significant

past, blood transfusion was the essential method in the treatment of renal anemia, whereas the transfusion requirement has recently lessened by the use of erythropoietin (EPO). Iron deficiency is frequent in patients with renal failure and iron need is increased by erythropoietin therapy; therefore, iron replacement is very important in the treatment of renal anemia. It is known that there is a debatable relationship between iron stores and HCV infection (5,6) as it is not clear whether HCV infection facilitates iron accumulation or increased iron storage predisposes to HCV infection. Further, the influence of HCV infection upon potential iron and erythropoietin therapy is controversial despite the frequent observation of high serum ferritin level and hepatosteatosis among patients with HCV infection that may depress the response to therapy (7). However, erythropoietin requirements and levels in HCV-positive and -negative patients were reported to be different in patients with end-stage renal disease (ESRD) (8,9).

In view of the recently published data reporting higher hemoglobin and hematocrit levels in HCV-positive compared to HCV-negative HD patients, we decided to compare the values of these two parameters in Egyptian HD population.

MATERIAL AND METHODS

The present retrospective study was carried out with a total of 99 subjects (68 male, 31 female, mean age 42.81±16.63 years) receiving chronic HD for at least one year in Mansoura Urology and Nephrology Center hemodialysis units, Mansoura , Egypt. Seventy patients were HCV positive and 29 were HCV negative as diagnosed by 3rd generation enzyme–linked immunosorbent assay (ELISA) and confirmed by PCR. (The Demographic, clinical and laboratory parameters are given in Table 1).

Laboratory

Anti HCV antibodies, Hepatitis B surface antigen and antibody as well as antibodies to human immunodeficiency virus (HIV) 1 and 2 were done on 3 monthly bases. All patients were subjected to monthly biochemical analysis for: Serum sodium, potassium, uric acid, fasting blood sugar, urea, creatinine, cholesterol, triglycerides, complete blood

Table 2. Hematological Parameters in HCV positive and HCV negative groups

Group/parameter	HCV- positive (70)	HCV-negative (29)	p value
Erythropoietin	39	16	ns
IV iron	10	10	ns
History of Blood transfusion	17	5	ns
L-Carnitine therapy	10	9	ns
Hemoglobin level (gm/dl)	10.32±2.03	10.22±1.52	ns
Hematocrit value	30.94± 6.089	30.77±4.53	ns
White blood cells (mm^3)	6.79±2.95	7.47±2.30	ns
Platelet count (mm^3)	218.4±30.28	239.71±83.06	ns
Erythropoietin dose IU/week	5000±2236	6250±2720	ns
IV iron dose (mg/week)	200±86.6	216.67±75.28	ns
Serum ferritin (ng/ml)	525.34±385.97	522.88±275.8	ns

ns: non significant

count and liver function test including alanine aminotransferase (ALT) levels were accepted. Sustained high ALT levels are considered if the last three values were >20 U/L. All samples were withdrawn from the patients before hemodialysis session.

Anti-HCV determinations were performed in all patients by third generation ELISA. HCV-RNA was detected in all positive patients by nested polymerase chain reaction (PCR) carried out with primers located with the 5`NC region of HCV-genome (Amplicor,Roche, Branchburg, NJ,USA) and repeated every 3 months.

Dialysis Prescription

Dialysis efficiency was calculated using Kt/V equation. All patients were dialyzed three times weekly using Fresenius Polysulphone capillary dialyser (F7) (UF- coefficient 6.4, surface area 1.6 m^2) aiming to achieve Kt/V of 1.3 at least. Exclusion criteria: Those who are not dialyzed at our unit at least 6 months before the start of the study, children <18 years old, those with acute blood loss, blood transfusion within 6 months, hepatitis B virus surface antigen–seropositive, autosomal dominant polycystic kidney disease and those with chronic pulmonary disease were excluded from the study

Erythropoietin therapy

Fifty – five patients were maintained on regular EPO therapy given subcutaneously after hemodialysis session. Twenty patients were on iron dextran therapy that was given by IV infusion during hemodialysis session.

Statistical analysis

It was performed using the statistical package for social studies (SPSS) for windows software package release 11. Results are presented as median and confidence intervals unless otherwise stated. Student t–test and Chi-squared test were applied as appropriate. A p value of ≤ 0.05 was considered significant. This study was approved by our local Institutional Research and Ethics board.

RESULTS

Clinical and Laboratory characteristics of HCV positive patients

Among 120 chronic HD patients who had been followed in our in-center HD units, 21 were excluded (6 patients had Adult Polycystic Renal disease, 8 were HBV positive and 7 had a history of blood transfusion in the previous 6 months) and the remaining 99 patients (68 male and 31 female) who had a mean age of 42. 81±16.63 years were the subject of this study. Seventy patients tested positive for HCV by third generation ELISA test that was confirmed by PCR giving a prevalence of 70.7% for HCV infection among our HD patients.

There were no statistically significant differences regarding age (40.41±14.17 vs 47.35±19.18 years, P=0.08), gender and hypertension prevalence between HCV-positive and HCV-negative patients. The mean HD duration was significantly longer in HCV-positive patients (66.54±43.92 months compared to HCV negative patients (30.96±23.17 years, P=0.006). Further, there was no statistically significant difference regarding all biochemical parameters studied except for ALT level which was significantly higher in HCV-positive patients (31.75±36.4 U/L) compared to HCV-negative patients (15.1±7.21 U/L, P=0.05) (Table 1).

Hematological parameters

As seen in table 2, there is no statistical difference between mean hemoglobin and hematocrit values in HCV-positive & HCV negative patients (10.32±2.03 gm/dl and 30.94± 6.09% &10.22±1.52 gm/dl vs 30.77± 4.53%, P=0.63 and 0.094, respectively).

Mean weekly EPO dose in HCV-positive patients (39 patients) was lower compared to HCV-negative patients (n=16) (5000±2236 vs 6250±2720 U/week, respectively). However this difference does not reach statistical significance (P=0.86). Also, there was no significant difference regarding weekly IV iron dose in HCV-positive compared to HCV-negative patients (200±86.6 vs 216.67±75.28 mg/week, P=0.7). Likewise, serum ferritin levels were comparable in both groups (525.34±385.97 vs 522.88±275.8 ng/ml respectively, P= 0.9).

DISCUSSION

In this study we noted that hemoglobin and hematocrit levels are comparable in HCV-positive and HCV-negative hemodialysis patients. Likewise was the weekly erythropoietin (EPO) dose, it was not substantially different despite the fact that it was lower in HCV-positive group.

In Egypt, HCV infection has reached an epidemic level of prevalence. We and others have reported a high prevalence (16%) of HCV antibodies among healthy blood donors and a much higher prevalence (38%) of HCV antibodies among patients with glomerulopathy (10,11). It is known that the prevalence of HCV is higher among HD patients than the general population (12) with an even higher prevalence -as high as 60% - of hepatitis C viremia reported among our renal transplant recipients (13).

In this study, a much higher prevalence (70.7%) of HCV viremia was documented among our HD patients and this strongly points to the magnitude of HCV problem among this selected group of patients. Our finding accords with a previous notion which indicated a generally much higher prevalence of HCV infection in developing countries among dialysis patients compared to that observed in the industrialized world (ranging from 8.5 to 75%) (14).

In addition to the number of blood transfusions, dialysis duration was reported as one of the most important risk factor for HCV acquisition on dialysis. The risk of acquiring HCV infection on HD has been estimated at 10% per year (15,16). This notion was supported by our observation that the mean HD duration was considerably longer in patients with anti-HCV antibody positive group compared to HCV-negative patients which is as well concordant with the literature data (17).

Anemia is the most common hematological abnormality in chronic renal failure. In the past, blood transfusion was the essential method in the treatment of renal anemia, whereas the transfusion requirement has recently lessened by the use of erythropoietin. It is known that the fetal liver is the primary site of production of the relevant haemopoietic hormones. These are the glycoproteins thrombopoietin and erythropoietin as well as and the somatomedins. After birth, the kidneys take over as the main site of EPO synthesis which is the primary regulator of erythropoiesis (18). Interestingly, the chronically diseased kidneys were reported to suppress the hepatic production of EPO by an unknown humoral mechanism and evidence for the latter concept has been provided in animal models of chronic renal failure (19). However, in patients with end-stage renal failure, serum EPO may increase after hepatitis B or C infection, resulting in an improvement of red cell status (20). The mechanism of this increase still needs to be identified.

On the contrary, hepatic EPO production increases under conditions of lowered oxygen supply. Apart from the effect of hypoxia, several agents may modulate EPO production in human hepatoma cultures similar to their effects in vivo. The EPO synthesis-increasing factors include IL-6, thyroid hormones, and vitamin A, while inhibition is exerted by the pro-inflammatory cytokines IL-1 and tumor necrosis factor-alpha (TNF-α) as well as by reactive oxygen species (21-24).

Compared with thrombopoietin, less interest has been paid to changes in EPO production related to acute or chronic non-malignant liver diseases. Recently, some reports demonstrated higher Hb and HCT levels in HCV-positive HD compared to HCV-negative patients. However the data discussing the effect of HCV infection in HD patients on both variables were controversial. While Sahin et al 2003 concluded from his study on 49 patients that higher Hb and HCT levels in HCV-positive compared to HCV-negative group was attributed most probably to increased production of EPO from HCV-infected patient's liver and this was supported by other case reports with improved red cell

status after HBV infection on maintenance HD (25-27). The improvement in RBC status after HBV/HCV infection was explained by the higher EPO levels as reported by Radovic et al 1999 (28). In contrast, Abdalla and associates in 2000 reported a higher EPO requirement in HCV-positive versus HCV-negative patients that were as a result of altered iron metabolism induced by chronic infection. They further concluded that the mechanism by which infection and inflammatory disease impair responsiveness to erythropoiesis is still poorly understood (29).

Our results indicate that HCV-positive Egyptian HD patients have comparable Hb and HCT levels to HCV-negative ones. This is in contrast to what has been reported previously (25-28). The lack of consistency between our and Sahin et al (2003) (25) results could be explained by: first the variations in patients' demography as our patients were relatively younger. Second, the HD duration was longer in our patients compared to the very short (12.5±9.0 months) one in the HCV-negative patients of Sahin's study (25) and this can partially explain their lower hemoglobin level. It was previously reported that dialysis duration is the only detrimental factor affecting the Hb/HCT values (29). Third explanation is the possible difference in viral genotyping. Although we did not test for HCV genotypes in this study, we have previously reported genotype 4 to be the predominant genotype among Egyptian population (30) and this may be partially responsible for the possible variation in endogenous erythropoietin production. Lastly, the degree of hepatic dysfunction we detected in this study as inferred from the higher serum ALT and AST levels in group of HCV-positive than in HCV-negative patients are concordant with another investigation (29) and might be influential as well.

Iron deficiency is frequent in patients with renal failure and iron need is further increased by EPO therapy; therefore, iron replacement is very important in the treatment of renal anemia (31,32). In addition, iron metabolism may be altered in patients with ESRD. However the influence of HCV infection upon parenteral iron and erythropoietin therapy is also controversial. In our study, HCV-positive patients required lower weekly EPO dose compared to HCV-negative group despite the fact that this difference was not substantial. Also, we did not observe a significant difference between both groups regarding their IV iron requirement. Our findings are in apparent harmony with the data of Altintepe L et al (8) who reported lesser EPO and iron requirements in HCV-positive HD compared to HCV-negative ones as a result of higher endogenous serum EPO concentrations and changes in iron metabolism in liver disease. However, Abdalla et al (9) reported a higher EPO requirement in HCV-positive HD patients. We can explain our finding of comparable weekly EPO and iron doses in HCV-positive patients by the assumption that even if endogenous EPO concentration is increased in them, resistance to EPO action could have occurred secondary to chronic infection which impairs iron availability or perhaps suppresses erythropoiesis by humoral factors, other cytokines or growth factors (33,34).

Our study would be more strengthened if we evaluated as well other factors that might affect the relationship between HCV infection and both hemoglobin as well as hematocrit levels such as phylogenic analysis for viral genotypes, viral titers and the degree of hepatic injury.

In conclusion, the results of our study allow us to conclude that the prevalence of HCV infection is higher in Egyptian hemodialysis patients than in general population and that hemoglobin and hematocrit levels are comparable in both HCV-positive and HCV-negative groups.

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