Research Article

Effect of hepatitis c virus on erythropoiesis in patients with end stage renal disease on regular haemodialysis

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ABSTRACT

INTRODUCTION: Hepatitis C virus infection is common among patients undergoing haemodialysis. Erythropoietin is a hormone that regulates erythropoiesis and is mainly produced by the kidneys. Previous studies demonstrated that regenerating hepatic tissue can produce more erythropoietin than normal hepatic tissue.

OBJECTIVE: To investigate the association of hepatitis C virus infection with endogenous erythropoietin level as well as haemoglobin and packed cell volume in patients on haemodialysis..

METHODS: This is a case-control study conducted on 70 patients with end stage renal disease on haemodialysis from 1st March to 1st September 2019. Patients grouped into anti-hepatitis C Virus IgG antibodies positive (case group), and anti-hepatitis C Virus IgG antibodies negative (control group). Serum levels of endogenous erythropoietin, duration of haemodialysis, haemoglobin level, packed cell volume, aspartate transaminase, alanine aminotransferase levels, platelets count and transferrin saturation were measures and compared between two groups.

RESULTS: Hepatitis C virus positive patients showed significantly higher haemoglobin, packed cell volume and transferrin saturation (10.19±1.79 mg/dL, 33.58% ±2.61 and 34.05%±2.38, respectively) than hepatitis C virus negative patients (9.42± 0.84 mg/dL, 31.31%±1.7 and 30.87%±4.04, respectively). In contrast, hepatitis C virus negative patients showed higher platelet count than hepatitis C virus positive patients (216.05×10^3/mL versus 190.53±49.07×10^3/mL) with a significant difference. The mean serum level of erythropoietin in hepatitis C virus positive patients was 10.74±4.14 mU/mL which was higher than that in hepatitis C virus negative patients (8.61±4.22 mU/mL) with a significant difference. In hepatitis C virus positive patients, erythropoietin showed a significant positive correlation with each of haemoglobin (r= 0.411, p= 0.036) transferrin saturation (r= 0.348, p= 0.039), and aspartate transaminase (r= 0.291, p=0.044)..

CONCLUSION: Anti-hepatitis C virus positive patients had higher levels of erythropoietin and haemoglobin and may require less erythropoietin and iron therapy as compared to anti- hepatitis C virus negative patients with end stage renal disease.

Key words: ESRD, HCV, Erythropoietin, Iraq.

INTRODUCTION

Hepatitis C virus (HCV) is one of the leading causes of acute and chronic hepatitis, cirrhosis of the liver, hepatocellular carcinoma, and related death.[1] Up to 1% of the world's population is estimated to be infected with HCV, corresponding to more than 71 million people with a viremic infection. The prevalence of HCV-specific antibodies in the general population varies from less than 1.5% in North America and some parts of South America to over 3.5% in some regions of Africa and Asia.[2]

Serological testing has demonstrated that HCV infection is highly prevalent among end stage renal disease (ESRD) patients undergoing haemodialysis (HD), and is a serious cause of increased morbidity and mortality in this group. Failures of HCV screening, excessive exposure to blood and blood products, nosocomial transmission of HCV in haemodialysis (HD) units, and long dialysis duration are the main determinants of increased risk of HCV infection in the HD patient group. [3] In the general population, 50%-90% of infected cases are asymptomatic. In 30% of cases of acute hepatitis, the resolution is spontaneous. Acute hepatitis resolves in 20%-30% of the cases spontaneously while in the vast majority it progresses to chronic hepatitis manifesting with variable, usually mild de-

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grees of inflammation and fibrosis.[4]

Erythropoietin (EPO) is a hormone produced by the kidney that promotes the formation of red blood cells in the bone marrow. During fetal life, EPO is primarily produced in the liver, but the main production shifts to the kidney after early neonatal life.[5] The majority of the non-renal erythropoietin production originates from the liver and has been estimated to be responsible for 10% of the total EPO synthesis in healthy controls, but can be up-regulated to 90–100% of the total EPO in response to anaemia, hypoxia, chronic kidney disease, or partial hepatectomy in rat models.[6]

This study aimed to investigate the association of HCV infection with endogenous erythropoietin level as well as haemoglobin, haematocrit platelets and liver function in patients with end stage renal disease on haemodialysis who are treated at the renal unit of Al-Imamain Al-Kadumain Medical City for six months in 2019.

METHODS

Study design & Setting: This is a hospital-based case-control study which was conducted on 70 patients with ESRD undergoing haemodialysis at Renal Unit in Al-Imamain Al-Kadumain Medical City during the period from 1st March to 1st September 2019.

Ethical consideration: The study was approved by the research committee and the administration of Al-Imamain Al-Kadumain Medical City. Verbal consent from each participant was obtained before data collection after explaining the aims of the study. Each patient was given the complete unconditioned choice to withdraw at any time. The confidentiality of data throughout the study was guaranteed and the patients were assured that data will be used for research purpose only.

Inclusion and exclusion criteria: We included adult patient over the age of 18 years who is already diagnosed with ESRD and treated by haemodialysis for at least one year and proved to have HCV.

Patients with a previous history of gastrointestinal bleeding or transfused blood in the previous six months had a history of active malignancy and/or haematopoietic disorders, had a history of polycystic kidney disease, or being pregnant, or receiving angiotensin II receptor blocker, Angiotensin-converting enzyme inhibitor (ACEi), interferon, ribavirin or erythropoietin were excluded from the study.

Procedure: Anti HCV antibodies were detected by enzyme-linked immunosorbent assay (ELISA). According to the results of anti-HCV IgG antibodies detected by ELISA. Those who tested positive for anti-HCV IgG antibodies were considered a case group, while those who tested negative for anti-HCV IgG antibodies were considered as a control group. All patients of both groups were subjected to full medical history and clinical examination including routine laboratory investigations.

Outcomes: Serum levels of endogenous erythropoietin was measured using a ready commercial kit (Elabscience/ China/ Catalog No: E-EL-H3640). The manufacturer's protocol was followed precisely. Data including age, gender, smoking status (never, ex, or current smoker), duration of HD, haemoglobin (Hb) concentration, haematocrit, aspartate transaminase (AST), alanine aminotransferase (ALT) levels, platelets count and transferrin saturation were collected through a direct interview or from patient's records. The results of these outcomes were compared between the two groups and suitable statistical tools were used to measure their statistical significance.

Statistical analysis: Data were analysed using IBM SPSS version 24 (SPSS Inc., Chicago, Illinois, USA). Numerical data were expressed as mean± standard deviation (SD) or median and range, as appropriate and analyzed with Student t-test. Categorical data were expressed as frequency and percentage. The Chi-square test (Fisher's exact test) was used to examine the relation between these variables. Multivariate logistic regression was used to find out the independent factors that are associated with the serum level of endogenous erythropoietin with a targeted Hb was 11-13 g%. The possible correlation between endogenous erythropoie-

Table 1| Demographic characteristics of the patients

Variables	HCV (+ve) N=30	HCV (-ve) N=40	P-value
Age in years			
Mean±SD	56.74±8.94	56.9±7.07	0.931
Range	34-69	38-70	
Gender			
Male	21(70%)	9(30%)	0.388
Female	24(60%)	16(40%)	
BMI, kg/m2	26.3±5.1	27.6±6.1	0.442
Duration of HD			
Mean±SD months	42.8±9.12	37.74±12.1	
Range months	13-64	10-56	0.229
Smoking			
Never	17(56.67%)	13(43.33%)	0.334
Ex/current smokers	18(45%)	22(55%)	

tin and other numerical variables was explored using Pearson's correlation parametric test. A p-value < 0.05 was considered significant.

RESULTS

The mean age of HCV positive patients was 56.74±8.94 years with a range of 34-69 years which almost similar to that of HCV negative patients that was 56.9±7.07 years with a range of 38-70 years with no statistical significance. Likewise, the gender distribution and smoking habit were comparable between the two groups with no significant difference. Mean BMI was slightly higher in HCV negative than HCV positive patients (27.6±6.1 kg/m^2 versus 26.3±5.1 kg/m^2); however, the difference was not significant. Patients positive for HCV had markedly higher mean of HD duration (42.8±9.12 months) than those HCV negative (37.74±12.1 months) with no significant difference (Table 1).

Multivariate logistic regression was used to test if serum EPO is independently associated with HCV infection. In this model, only three factors showed significant association with HCV. Hb concentration was ≥10 mg/mL in 56.67% in HCV positive patients compared with only 17.5% of HCV negative patients who had such concentration, with a significant difference (OR=4.64, 95%CI=1.16-18.5, p= 0.030). Likewise, the majority of HCV positive patients (86.67%) showed serum transferrin saturation ≥ 30% compared to 57.5% of HCV negative patients, with a significant difference (OR= 4.78, 95%CI=1.03-22.16, p= 0.045). Finally, serum EPO was ≥ 9.5 mU/l in 50 HCV positive patients, while only 27.5% of HCV negative patients had such a level, with a significant difference (OR= 2.31, 95%CI=1.04-13.92, p= 0.042) as shown in table 2. Pearson's correlation was used to explore the possible correlation between EPO level and other quantitative variables in HCV positive and HCV

Table 2 | Multivariate logistic regression

Variables		HCV+ (N=30)	HCV- (N=40)	p-value	OR(95%CI)
Hb mg/dL	**< 10**	13 (43.33%)	33(82.5%)	0.030	1.0 CI 4.64(1.16-18.5)
	≥ 10	17 (56.67%)	7(17.5%)		
Haematocrit %	**< 30**	6 (20%)	11(27.5%)	0.118	1.0 CI 1.78(0.1-6.14)
	≥ 30	24 (80%)	29(72.5%)		
Transferrin saturation %	**< 30**	4 (13.33%)	17(42.5%)	0.045	1.0 CI 4.78(1.03-22.16)
	≥ 30	26 (86.67%)	23(57.5%)		
Platelets (×10^3/mL)	**< 200**	20 (66.67%)	16(40%)	0.079	1.0 CI 0.32(0.09-1.14)
	≥ 200	10 (33.33%)	24(60%)		
AST U/L	**< 30**	6 (20%)	20(50%)	0.21	1.0 CI 3.08(0.53-17.94)
	≥ 30	24 (80%)	20(50%)		
ALT U/L	**< 35**	7 (23.33%)	22(55%)	0.239	1.0 CI 1.32(0.26-6.79)
	≥ 35	23 (76.67%)	18(45%)		
EPO mU/mL	**< 9.5**	15 (50%)	29(72.5%)	0.042	1.0 CI 2.31(1.04-13.92)
	≥ 9.5	15 (50%)	11(27.5%)		

Table 3 | Pearson's correlation for EPO and other quantitative variables in HCV+ and HCV- patients under haemodialysis

Variable	HCV+		HCV-	
	r	p-value	r	p-value
Age	0.091	0.491	0.101	0.312
BMI	0.129	0.237	0.097	0.311
Duration of HD	0.219	0.072	0.188	0.112
Hb	0.411	0.036	0.209	0.11
Platelets	-0.108	0.372	-0.137	0.209
Hematocrit	0.302	0.082	0.284	0.092
Transferrin saturation	0.348	0.039	0.327	0.039
AST	0.291	0.044	0.199	0.098
ALT	0.274	0.069	0.211	0.121

negative groups. The results are demonstrated in table 3. In HCV+ patients, EPO showed a significant positive correlation with each of Hb (r= 0.411, p= 0.036) transferrin saturation (r= 0.348, p= 0.039), and AST (r= 0.291, p=0.044). In HCV- patients, there was only one positive significant correlation between EPO and transferrin saturation (r= 0.327, p= 0.039).

DISCUSSION

One of the most important findings in the present study was that anti-HCV-positive patients portrayed significantly higher levels of Hb and packed cell volume (PCV) than anti-HCV-negative patients. For, Hb this association stills significant even in multivariate regression which indicates that Hb is independently linked with HCV infection. These results are in accordance with most previous studies worldwide. In the USA,[7] retrospectively the records of 76 patients with HCV infection and 57 patients free from this infection. The study found that mean Hb was 12.6±1.2 g/dl and 11.9±1.1 g/dl respectively, while PCV was 39% and 37.43%, respectively, with a statistical significance. Almost similar results were reported among Egyptian patients,[8] Turkish patients[9] and Saudi patients.[10] However, in a Tunisian study on 57 patients, the authors did not find a significant difference in Hb or PCV between anti-HCV-positive patients and anti-HCV-negative patients, probably due to the small sample size.[11]

Such elevation in Hb and PCV in anti-HCV-positive patients has been proposed to be either due to the secretion of erythropoietin from regenerating liver cells[12] or due to alternations in iron metabolism.[13]

We found elevated transferrin saturation in anti-HCV-positive patients compared to anti-HCV-negative patients independent of other factors. In accordance, a Turkish study has revealed that anti-HCV-positive patients had far higher transferrin saturation than anti-HCV-negative patients (46.9±24.1 vs. 24.7±11.6).[14] This finding was further supported by the Egyptian study in which the transferrin saturation was 49±41 and 40±54 in patients with positive and negative for anti-HCV antibodies, respectively[8] as well as by Kalantar-Zadeh et al.[15] among American patients.

We also found a decline in platelets count in anti-HCV-positive patients compared to anti-HCV-negative patients. This agrees with many studies which confirmed the reduction of platelets in patients with HCV whether on HD or not. A systematic review included 27 studies with a total of 11641 patients estimated the average prevalence of thrombocytopenia in chronic HCV infection to be near 24%.[16]

Each of ALT and AST were found to be significantly elevated in anti-HCV-positive patients than anti-HCV-negative patients in the current study which is in the context of almost all previous studies in this issue.[8,17,18] However, in multivariate analyses, these enzymes were no more associated with HCV infection which indicates that the liver enzyme may associate with other factors such as age or duration.

In this study, endogenous erythropoietin in HCV+ patients was higher than that in HCV- patients even in multivariate analysis. Such a result is supported by that of many studies. In USA, Altintepe et al[19] has revealed that endogenous EPO was significantly higher in HCV positive patients (9.34 mU/ml) than in HCV negative patients (3.59 mU/ml). Similar results were reported in another USA study,[7] Egyptian study,[8] and Turkish study.[9] Thus, there was almost a consensus about the role of HCV infection in increasing the serum level of endogenous EPO in a patient on HD.

CONCLUSION

Anti-HCV-positive patients on HD portrayed significantly higher levels of Hb and PCV and transferrin saturation than anti-HCV-negative patients, while the reverse is true for platelets count; Serum level of ALT and AST remains within normal range although anti-HCV-positive patients had higher levels than anti-HCV-negative patients; Erythropoietin level is significantly higher in anti-HCV-positive patients as compared to anti-HCV negative HD patients and correlates significantly with Hb, transferrin saturation and AST.

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Abbreviations list: Alanine aminotransferase (**ALT**), Angiotensin converting enzyme inhibitor (**ACEi**), Aspartate transaminase (**AST**), Body mass index (**BMI**), End stage renal disease (**ESRD**), Enzyme linked immunosorbent assay (**ELISA**), Erythropoietin (**EPO**), Gram pe square metre (**kg/m²**), Haemodialysis (**HD**), Haemoglobin (**Hb**), Hepatitis C virus (**HCV**), Milligram per millilitre (**mg/mL**), Milliunit per litre (**mU/l**), Packed cell volume (**PCV**), Standard deviation (**SD**), Statistical Package for the Social Sciences (**SPSS**).

Conflict of interest: Authors have nothing to declare.

Funding: Nothing apart from personal fund.