

## HCV Infection And Erythropoietin In Haemodialysis Patients

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### ABSTRACT

**Background:** Renal anemia is associated with increased cardio vascular morbidity and mortality and decreased quality of life. It may worsen the preexisting heart disease and consequently may accelerate the progression of renal dysfunction in a vicious circle. Renal anemia related mainly to decreased production of erythropoietin (EPO) by the kidney, decreased its response by bone marrow and disturbed iron status. **Aim of the Study:** Was to evaluate the relationship between HCV infection and erythropoietin in patients on regular HD. **Patients and Methods:** This study included 50 hemodialysis patients (25 patients +ve HCV and 25 -ve HCV), 16 were male and 34 were female, and their ages ranged from 20 to 67 years, with a mean  $\pm$  SD of  $50.5 \pm 12.7$  years, 15 apparently healthy individuals with normal kidney function and not anemic were included in the study as a control Group. **Results:** The patients were divided into two groups as the following: **Group I** consisted of 25 hemodialysis patients were positive for HCV antibody, which was confirmed by PCR, and their ages ranged from 27 to 67 years, with a mean of  $47.28 \pm 11.91$  years. **Group II** consisted of 25 hemodialysis patients were negative for HCV antibody; their ages ranged from 20 to 65 years, with a mean age of  $46.32 \pm 11.50$  years. In contrast, 15 patients served as age-matched and gender-matched; their ages ranged from 20 to 55 years, with a mean age of  $44.13 \pm 10.76$  years, and were used as controls. **Conclusion:** Serum levels of erythropoietin correlate with chronic hepatitis C infection, which was associated with an increased number of RBCs and higher values of hemoglobin and hematocrit, which Result in lowering of the necessary erythropoietin dose and iron therapy.

**Keywords:** HCV Infection, Erythropoietin, Regular Hemodialysis

### INTRODUCTION

Chronic kidney disease (CKD) is a public health problem and one of the most important cause of death worldwide <sup>(1)</sup>.

Anemia is an almost constant complication of advanced renal failure, and progress as the CKD progress. Renal anemia is associated with increased cardio vascular morbidity and mortality and decreased quality of life. It may worsen the preexisting heart disease and consequently may accelerate the progression of renal dysfunction in a vicious circle <sup>(2)</sup>.

Renal anemia related mainly to decreased production of erythropoietin (EPO) by the kidney, decreased its response by bone marrow and disturbed iron status <sup>(3)</sup>.

Erythropoietin is a glycoprotein hormone that regulates erythropoiesis and is mainly produced by the kidney. Epo deficiency is the predominant factor in renal anemia. With the development of recombinant human erythropoietin (rHuEpo) in 1989 new era of treatment of renal anemia started. Replacement

of erythropoietin with rHuEpo results in an increase in red blood cell mass and reduced the need for blood transfusion <sup>(4)</sup>.

Considerable percentage of patients on regular HD is already infected with hepatitis C virus and is HCV antibody positive. Incidence of HCV infection increased as the duration of dialysis increased. The risk of acquiring HCV infection on HD is approximately 10% per year <sup>(5)</sup>.

The level of hemoglobin is higher in infected patients than in non-infected group <sup>(6)</sup>. The proposal was that this association may be due to secretion of erythropoietin from regenerating liver cells or due to alteration in iron metabolism <sup>(4)</sup>. Several case report have linked hepatic regeneration secondary to viral or toxic injury with increased red cell production in patients with and without CKD. Interestingly, patients with HCV antibodies positive had higher erythropoietin levels.

### AIM OF THE WORK

## HCV INFECTION AND ERYTHROPOIETIN IN HAEMODIALYSIS PATIENTS

Is to evaluate the relationship between HCV infection and erythropoietin in regular HD patients.

### PATIENTS AND METHODS

This study was designed to assess the influence of chronic hepatitis C infections on erythropoiesis in chronic hepatitis C hemodialysis patients and was carried out in the Nephrology Unit, and the Internal Medicine Department, at El Hussein Hospital, ALAzhar University, in the year 2017 and it included **50** hemodialysis patients (25 +ve HCV and 25 -ve HCV). Of them, 16 were male and 34 were female, and their ages ranged from 20 to 67 years, with a mean  $\pm$  SD of  $50.5 \pm 12.7$  years. In contrast, 15 apparently healthy individuals with normal kidney function and not anemic were included in the study. All of them had been on regular hemodialysis for at least 6 months, 4 hours/day, three times a week.

All patients provided informed consent permitting data sampling and analysis at the time of initiation of the dialysis therapy. The protocol for the study was approved by the Ethics Committee of ALAzhar University, including outside experts.

Patients were labeled as HCV-positive if they had positive anti-HCV antibodies on two separate occasions or on one occasion, along with a confirmatory HCV polymerase chain reaction testing.

**The patients were divided into two groups as the following:**

**Group I** consisted of **25** hemodialysis patients were positive for HCV antibody, which was confirmed by PCR, and their ages ranged from 27 to 67 years, with a mean of  $47.28 \pm 11.91$  years.

**Group II** consisted of **25** hemodialysis patients were negative for HCV antibody; their ages ranged from 20 to 65 years, with a mean age of  $46.32 \pm 11.50$  years.

In contrast, **15** patients served as age-matched and gender-matched; their ages ranged from 20 to 55 years, with a mean age of  $44.13 \pm 10.76$  years, and were used as controls.

Patients with CKD on regular HD either hepatitis HCV positive or negative,

### RESULTS

**Table 1:** Comparison between patients group according to erythropoietin level

Erythro	Group I (+ve HCV) (N=25)	Group II (-ve HCV) (N=25)	Group III(Control) (N=15)	ANOVA	p-value
Mean $\pm$ SD	1.30 $\pm$ 0.81	0.76 $\pm$ 0.78	0.65 $\pm$ 0.35	2.978	0.014
Range	0.1-7	0.02-3.40	0.1-1.2		

This table shows statistically significant difference between groups Regarding erythropoietin level.

having iron deficiency anemia were included in the study.

Patients with a history of repeated blood transfusion or massive blood loss in the last 6 months, Pts with polycystic kidney, Pts with cryoglobulinemia, Pts with active malignancy, Pts with hemopiotic disorder, Pts with gastrointestinal bleeding and Pts undergoing active treatment with interferon or ribavirin and borderline positive tests or with a history of HCV with negative serologic tests were excluded from the study.

Informed consent was obtained with the approval of local ethical committee.

All patients were subjected to the following: History taking stressing on age, sex, duration of HD, blood transfusion, treatment received especially for anemia and antivirals. Laboratory investigations including: CBC, FBS, ESR, S IRON, TIBC, Tsat, S ferritin, Bl urea, S creatinine, ALT, AST, HCV Abs by ELISA and S Erythropoietin by ELISA. Blood samples were collected from each participant by venipuncture in empty centrifuge tubes: incubated in water bath at 37°C for 15 minutes then centrifuged at 3500 rpm. Sera were separated, divided into aliquots and stored at -80°C till use. Hemolysed samples were discarded.

The quantitative detection of erythropoietin in serum levels was performed using a commercially available ELISA kit provided by EIAab following the manufacture recommendations.

If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 30 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 750 mL of Wash Buffer.

### Statistical analysis

Data were analyzed using Statistical Program for Social Science (SPSS) version 20.0. Quantitative data were expressed as mean $\pm$  standard deviation (SD). Qualitative data were expressed as frequency and percentage.

**Table 2:** Comparison between group I and group II Regarding erythropoietin level

Erythro	Group I (+ve HCV) (N=25)	Group II (-ve HCV) (N=25)	t-test	p-value
Mean±SD	1.30±0.81	0.76±0.78	2.401	0.020
Range	0.1-7	0.02-3.40		

This table shows statistically significant difference between groups according to erythropoietin level.

**Table 3:** Comparison between patients groups Regarding hemoglobin

Hb.	Group I (+ve HCV) (N=25)	Group II (-ve HCV) (N=25)	Group III (Control) (N=15)	ANOVA	p-value
Mean±SD	10.97±1.40	9.40±1.48	12.27±1.13	20.240	<0.001
Range	8.5-13.5	7-13	10.5-14		

This table shows highly statistically significant difference between patients groups according to Hemoglobin.

**Table 4:** Comparison between group I and group II Regarding hemoglobin level

Hb.	Group I (+ve HCV) (N=25)	Group II (-ve HCV) (N=25)	t-test	p-value
Mean±SD	10.97±1.40	9.40±1.48	3.853	<0.001
Range	8.5-13.5	7-13		

This table shows highly statistically significant difference between patients groups according to Hemoglobin.

**Table 5:** Comparison between group I and group II Regarding S. ferritin

S. Ferritin	Group I (+ve HCV) (N=25)	Group II (-ve HCV) (N=25)	t-test	p-value
Mean±SD	94.68±68.72	21.18±4.10	5.338	<0.001
Range	20-250	10-30		

This table shows statistically significant difference between patients groups according to serum ferritin.

**Table 6:** Comparison between patients groups Regarding treatment received

	Group I (+ve HCV) (N=25)	Group II (-ve HCV) (N=25)	Group III (Control) (N=15)	Chi-square test	p-value
<b>Bl. Trans. (3ms)</b>					
No	21 (84.0%)	15 (60%)	15 (100.0%)	6.274	0.043
Yes	4 (16.0%)	10 (40%)	0 (0.0%)		
<b>Take Erythro</b>					
No	13 (52.0%)	7 (28%)	15 (100.0%)	23.909	<0.001
Yes	12 (48.0%)	18 (72%)	0 (0.0%)		
<b>Treatment Hepatitis</b>					
No	23 (92.0%)	25 (100.0%)	15 (100.0%)	3.059	0.217
Q urevo	2 (8.0%)	0 (0.0%)	0 (0.0%)		

This table shows statistically significant difference between groups according to receiving blood transfusion, taking erythropoietin and receiving DAAs.

## DISCUSSION

Chronic kidney disease (CKD) is a public health problem. It is one of the most important causes of death worldwide and also a major cause of anemia in developing countries. The long-term survival and good quality of life of patients with chronic renal failure (CRF) depend, among other factors, on hemoglobin, iron status, and bone marrow response to erythropoiesis stimulating agents. In general, anemia is normocytic, normochromic, and usually well tolerated until the advanced stages of kidney disease, whereas other factors may

contribute to its establishment, maintenance, or aggravation<sup>(3)</sup>.

Anemia is an almost constant complication of advanced renal failure, which might worsen pre-existing heart disease and, as a consequence, accelerate the progression of renal dysfunction. Erythropoietin, which is mainly produced by the kidneys, is a glycoprotein hormone that regulates red blood cell production or erythropoiesis<sup>(5)</sup>. The high incidence of anemia in chronic kidney disease is due predominantly to inadequate production of erythropoietin from failing kidneys. The

specific treatment of choice for anemia due to chronic renal failure is recombinant human erythropoietin (rHuEPO), a drug considered a historic milestone since the time of its clinical use and a major therapeutic advancement in the treatment of anemia due to chronic renal failure. Erythropoietin remains a cornerstone in the treatment of anemia of renal failure<sup>(4)</sup>. Replacement of erythropoietin with rHuEpo can increase red blood cell mass and reduce the need for blood transfusions<sup>(7)</sup>.

Hepatitis C virus (HCV) infection causes significant morbidity and mortality in patients with end-stage renal disease on hemodialysis. The prevalence of anti-HCV antibody is higher in patients on hemodialysis (HD) than in healthy populations. Several risk factors contributing to this high prevalence of HCV infection have been identified, and include the number of blood transfusions, duration of end-stage renal disease (ESRD), mode of dialysis, and prevalence of HCV infection in the dialysis unit<sup>(8)</sup>.

Patients in maintenance hemodialysis are at increased risk of acquiring HCV infection and consequently have a higher prevalence than general population<sup>(9)</sup>, but large variations in HCV infection occur among dialysis units in different geographic areas. For example, the prevalence of HCV infection among dialysis patients in different European and Mediterranean countries varied between 2% and 63%<sup>(10)</sup>.

Study for influence of chronic HCV on anemia in dialysis patients remains inconclusive. Lessened anemia was observed in chronic HCV positive hemodialysis patients, which demanded less erythropoietin dose than in HCV negative hemodialysis patients<sup>(8)</sup>. On the other hand, increased endogenous erythropoietin production was reported in hemodialysis patients with hepatitis virus infections<sup>(11)</sup>.

The aim of our study was to evaluate the effect of HCV infection on erythropoiesis in patients with end-stage renal disease on regular hemodialysis through our patient population.

In the current study, total of 50 hemodialysis patients were divided into (25 patients with HCV infection and 25 patients without HCV infection). In the HCV-positive group (Group I), the mean age was  $47.28 \pm 11.91$  years; their ages ranged from 27 to 67 years. In the HCV-negative group (Group II) the mean age was  $46.32 \pm 11.50$  years; their

ages ranged from 20 to 65 years. The mean age was  $44.13 \pm 10.76$  years in controls group; their ages ranged from 20 to 55 years. A slightly higher percentage of patients on regular hemodialysis were female.

All patients provided informed consent permitting data sampling and analysis at the time of initiation of the dialysis therapy. The protocol for the study was approved by the Ethics Committee of AlAzhar University, including outside experts. All of them had been on regular hemodialysis for at least 6 months, 4 hours/day, three times a week.

Statistically, there was no significant difference in age and sex between the studied groups. This finding was similarly with the result in the study done by SAIFAN *et al.*<sup>(12)</sup>.

The present study highlights the duration of dialysis as an important risk factor for infection among hemodialysis patients. In our study, we also observed that the mean duration of time of hemodialysis was significantly longer in Group I ( $8.76 \pm 2.73$  years) than in Group II ( $7.36 \pm 2.72$  years). This difference was not statistically significant ( $P = 0.086$ ). This observation was in agreement with previous reports in Palestine, Moldavia, and other studies from different regions of the world<sup>(13)</sup>.

It has been reported that the duration of hemodialysis is significantly longer in anti-HCV-positive patients than in anti-HCV-negative patients. Further, it has been observed that patients on hemodialysis for more than 10 years have an increased incidence of HCV infection. The risk of acquiring HCV infection on hemodialysis is estimated at approximately 10% per year<sup>(14)</sup>. In the study of Saifan *et al.*<sup>(12)</sup>, observed that patients with HCV infection spent a significantly longer time on hemodialysis than those without HCV infection.

Also, Altintepe *et al.*<sup>(11)</sup> reported that 49 hemodialysis patients had significantly higher serum endogenous erythropoietin levels in HCV (+) patients than HCV (-) HD patients ( $9.43 \pm 6.47$  mU/ml vs  $3.59 \pm 2.08$  mU/ml,  $p = 0.008$ ).

In the present study, we demonstrated that hemodialysis patients with HCV infection tended to have higher mean hemoglobin levels than other groups. These results are generally compatible with other studies<sup>(8,15)</sup>. Those observed patients with HCV infection were found to have higher hemoglobin levels compared with HCV-negative patients.

Similarly, *Sahin et al.* <sup>(5)</sup> found that anti-HCV-positive patients with end-stage renal disease had higher hemoglobin and hematocrit levels than anti-HCV-negative patients.

A highly significant association of hemoglobin with HCV-positive group and the relation between HCV-positive group and erythropoiesis has been proposed to be either due to the secretion of erythropoietin from regenerating liver cells <sup>(4)</sup> or due to alternations in iron metabolism <sup>(16)</sup>.

High serum ferritin levels have been found to be associated with increased hospitalization, and a recent rise in serum ferritin is reported to be an imminent death risk in maintenance hemodialysis patients. An association between dialysis morbidity, including risk for infection, and iron overload reflected by a high serum ferritin also has been reported <sup>(17)</sup>.

The mean serum creatinine value was  $6.97 \pm 2.10$  mg/dL in HCV-positive patients,  $6.05 \pm 1.14$  mg/dL in HCV-negative patients, and  $0.65 \pm 0.35$  in controls group. Serum creatinine levels showed a significantly increase in HCV-positive patients and HCV-negative patients on regular hemodialysis as compared by controls group ( $p < 0.001$ ). These data were in accordance with data of *Ul Amin et al.* <sup>(18)</sup>; *Abumwais et al.* <sup>(19)</sup> and *Kosaraju et al.* <sup>(20)</sup>.

In the current study, the mean urea value was  $106.28 \pm 19.43$  in HCV-positive patients,  $130.14 \pm 18.58$  in HCV-negative patients, and  $29.93 \pm 7.16$  in controls group. Serum urea levels showed a significantly increase in HCV-negative patients on regular hemodialysis compared with HCV-positive patients on regular hemodialysis and controls group ( $p < 0.001$ ). These data were in accordance with the results of *Ul Amin et al.* <sup>(18)</sup>.

The current study presented correlation between serum erythropoietin, Hb and serum Ferritin with other parameters, using Pearson Correlation in HCV-positive patients. Our findings showed serum erythropoietin was significantly positive correlated to the age and TIBC in all cases of the HCV-positive group, were as  $r = 0.416$ ;  $p = 0.039$  and  $r = 0.514$ ;  $p = 0.009$  respectively. On the other hand, Hb count was significantly positive correlation with the age, TIBC, and ALT, were as  $r = 0.433$ ;  $p = 0.031$ ,  $r = 0.466$ ;  $p = 0.019$  and  $r = 0.391$ ;  $p = 0.053$  respectively. However, Hb was

significantly negative correlated to Transferrin saturation ( $r = -0.545$ ;  $p = 0.005$ ). Additionally, serum Ferritin had strong positive correlated to TIBC ( $r = 0.667$ ;  $p < 0.001$ ). Our findings were in accordance with those studies <sup>(1,5,21)</sup>.

The current study presented correlation between serum erythropoietin, Hb and serum Ferritin with other parameters, using Pearson Correlation in HCV-negative patients. Our findings showed Hb count was significantly positive correlated to TIBC in all cases of the HCV-negative group ( $r = 0.419$ ;  $p = 0.38$ ).

In pure financial terms, this may appear to translate to cost savings on erythropoietin at the dialysis center and to avoid the deleterious effect of hemoglobin levels  $> 11$  g/dL because of the risk of death and major cardiovascular events <sup>(22)</sup>.

## CONCLUSION

Serum levels of erythropoietin correlate with chronic hepatitis C infection, which was associated with an increased number of RBCs and higher values of hemoglobin and hematocrit, resulting in lowering of the necessary erythropoietin dose and iron therapy. Hepatitis C infection in hemodialysis patients tends to have higher baseline hemoglobin and decreased need for erythropoietin therapy. We believe that further investigations are needed to clarify the role of HCV on erythropoiesis in patients on hemodialysis.

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