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RESEARCH ARTICLE

Value of serum Erythropoietin level in patients with and without anemia associated with hepatitis C antiviral treatment

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Abstract

Background : Erythropoietin (EPO) is a glycoprotein hormone that regulates red blood cells production (erythropoiesis). It is commonly used in anemia which occurs in approximately 10% up to 30% of patients receiving hepatitis C antiviral therapy. **Aim of the study:** The aim of this work is to determine the value of serum EPO level in patients with and without anemia associated with hepatitis C antiviral treatment{pegylated interferon(PEG-INF) and ribavirin (RBV)} to clarify its role in those patients.

Subjects and methods: This study included 75 subjects who were subdivided into the following subgroups; Group I; 15 non-anemic chronic HCV patients and not receiving anti-viral therapy, Group II; 15 anemic chronic HCV patients and not receiving anti-viral therapy, Group III; 15 non-anemic chronic HCV patients and under anti-viral therapy, Group IV; 15 anemic chronic HCV patients and under anti-viral therapy and Control group; 15 healthy volunteers with matched age and sex. All studied individuals were subjected to detailed history taking, thorough physical examination, abdominal ultrasonography and routine laboratory investigations before combined treatment with PEG-INF and RBV for HCV. Estimation of EPO in serum was done by IMMULITE.

Results: This study revealed that serum EPO level didn't show significant difference as regards gender (28.54±11.83 in males Vs 29.81±11.44 in females, P-value = 0.68). Statistically significant higher levels of serum EPO was found in patients when compared to controls and in treated patients when compared to untreated patients (P- value < 0.001). The highest levels were found in Group IV, while the lowest levels were found in Group I. Negative correlation was found between serum EPO and HB% levels in anemic patients (treated or untreated).

Conclusion: Chronic liver disease by HCV does not affect the physiological EPO response to anemia. Serum EPO level was significantly higher in chronically infected patients with HCV compared with healthy control group. Also, serum EPO level increased in anemic more than non-anemic patients specially when treated with PEG-INF and RBV.

Introduction

Erythropoietin is a glycoprotein hormone that regulates red blood cell production (erythropoiesis) by binding to receptors on committed progenitor cells in the bone marrow and other hematopoietic tissues, resulting in proliferation and terminal maturation of erythroid cells **[1]**.

Erythropoietin is primarily synthesized in the kidneys by peri-tubular fibroblast. Liver is the main extra-renal site of EPO production, around 10% - 20% of the circulating EPO is produced by hepatocytes, Kupffer cells and other cells as lipocytes, stellate cells or perisinsoidal cells **[2]**.

Combination therapy for HCV infection with PEG-INF and RBV can be associated with a potentially dose-limiting haemolytic anemia. Correcting or preventing the anemia by modifying the dose of RBV is not an optimum approach because it can significantly compromise sustained virologic response (SVR) rates **[3]**.

Erythropoietin concentrations are inversely related to hemoglobin concentration, ranging from approximately 10 mU/mL in non anemic conditions up to 10,000 mU/mL in severe anemia **[4]**. So, this study was conducted to determine the value of serum EPO level in patients with and without anemia associated with hepatitis C antiviral treatment to clarify its role in those patients.

Subjects and methods:

This study was carried out in Internal medicine, Tropical medicine and Clinical pathology departments, faculty of medicine, Zagazig university during the period from September 2011 to October 2012.This study included 75 subjects who were divided into 4 patient groups with matched child scores and a control group :

Group I : 15 chronic hepatitis C patients who were non-anemic and not receiving anti-viral therapy.

Group II : 15 chronic hepatitis C patients who were anemic and not receiving anti-viral therapy.

Group III : 15 chronic hepatitis C patients who were non-anemic and under anti-viral therapy with RBV and PEG-INF.

Group IV : 15 chronic hepatitis C patients who were anemic and under anti-viral therapy with RBV and PEG-INF

Control group : 15 healthy volunteers with matched age and sex.

After an informed consent, all the studied individuals were subjected to detailed history taking, thorough physical examination, abdominal ultrasonography and laboratory investigations including {CBC, LFT, KFT, fasting blood sugar (FBS), Anti HCV antibodies, PCR for HCV RNA, HBVs Ag, α-Fetoprotein (AFP) and antinuclear antibodies (ANA). In addition, EPO serum level was estimated by IMMULITE which is a solid-phase, chemi-iluminescent immune-metric assay system **[5]**.

Exclusion criteria:

Patients chronically infected with HCV with liver cirrhosis, other causes of hepatitis rather than HCV, renal failure, hepatocellular carcinoma (HCC), cardiac diseases, lung diseases, smokers, hypertension, diabetes mellitus, other causes for tissue hypoxia rather than anemia or bone marrow diseases were all excluded from this study.

Statistical analysis:

Data were collected and analyzed with Special Package for Social Science (SPSS) version 20. Data were expressed using descriptive statistic (mean and standard deviation, median and range, frequency and percentage and were analyzed using "t" test and chi-square tests. One way Analysis Of Variance (ANOVA) was done to compare different parameters between more than two groups. Correlation was done using Pearson correlation test. P-value < 0.05 was considered as significant and P-value < 0.001 was considered as highly significant.

Results:

Table (1): Regarding age and sex, there was no statistically significant difference between the studied groups (P-value was 0.85 and 0.79, respectively).

Table (2) showed that there were high significant differences between the studied groups as regards HB%, RBCs and serum albumin (P-value < 0.001), and significant differences between the studied groups as regards SGPT, SGOT, PT, α-Fetoprotein and blood urea (P-value <0. 05). While no significant differences were found between the studied groups as regards WBCs, platelets, total bilirubin, serum creatinine and FBS (P-value > 0.05).

Table (3) showed that serum EPO level differed significantly among the studied groups of patients (P-value < 0.001). The highest levels were found in Group IV, while the lowest levels were found in the control group.

Table (4) showed that there was a significant positive correlation between serum EPO level and the following parameters: Serum albumin, AFP and blood urea, while a significant negative correlation was recorded between serum EPO level and HB. No significant correlation was found between serum EPO level and the following parameters: RBC_S, WBC_S, platelets, SGPT, SGOT, total bilirubin, PT, serum creatinine and FBS.

Table (5) showed that there were significant differences in comparing any of the studied groups of patients as regards serum EPO level except for Group II and Group III; there was no significant difference between them.

Table (6) showed that there was a highly significant difference of serum EPO level between patients and control group (P-value < 0.001). Also there was a highly significant difference of serum EPO level between treated and untreated patients (P-value < 0.001). Treated patients had a higher serum EPO level than untreated.

Table (1) : Demographic data of studied patient groups and control group.

Item	Control group		Group I		Group II		Group III		Group IV		P value
Age (year)											
Range	21-61		28-53		29-52		29-53		32-56		0.85
Mean ± (SD)	41.26±13.45		41.33± 8.13		41.53 ±6.65		40.93 ±7.85		44.20±6.03		NS
	No	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**P value**
Gender											
Male	8	53.3	9	60.0	9	60.0	11	73.3	8	53.3	0.79
Female	7	46.7	6	40.0	6	40.0	4	26.7	7	46.7	NS

Table (2): Laboratory data of the studied groups.

Item	Control group	Group I	Group II	Group III	Group IV	P value
HB (g/dl)	13.4±8	13.3±0.87	10.3±0.6	13.3±0.9	10.1 ± 0.7	<0.001 HS
RBCs ($x10^3/mm^3$)	4.8±0.5	4.6±0.4	3.8±0.3	4.8±0.3	3.8 ± 0.2	<0.001 HS
WBCs ($x10^3/mm^3$	5.5±0.8	5.2±0.9	5.6±1.3	4.9±0.6	4.5±0.8	0.079 NS
Platelet ($x10^3/mm^3$)	298.2±54.6	202.0±33.3	242.3±49.6	218.4±51.2	163.6±15.7	0.051 NS
SGPT (U/L)	19.4±5.2	39.4±14.7	34.4±14.7	40.0±23.7	38.4±22.23	0.009 S
SGOT (U/L)	21.6±4.1	37.0±14.9	34.6±13.7	39.4±24.2	38.3±22.6	0.044 S
Total Bilirubin (mg/dL)	0.72±0.18	0.92±0.36	1.14±0.6	0.88±0.32	0.98±0.36	0.069 NS
Albumin (g/L)	4.08±0.39	4.32±0.55	4.46±0.38	4.74±0.28	4.61±0.25	<0.001 HS
PT (Seconds)	11.6±0.5	12.7±1.4	13.1±1.3	12.6±1.2	13.2±1.1	0.04 S
AFP	32.7±6.4	32.8±8.1	37.7±4.2	39.5±7.1	39.9±4.9	0.002 S
Blood urea (mg/dL)	31.4±4.6	30.4±5.2	34.6±5.5	35.2±5.4	36.6±4.5	0.006 S
Serum creatinine (mg/dL)	0.85±0.16	0.83±0.22	0.87±0.24	0.91±0.17	0.90±0.20	0.865 NS
FBS	93.2±8.2	95.3±17.3	102.6±16.4	104.1±27.5	104.1±27.5	0.253 NS

Table (3): Serum EPO level (mU/ml) among studied groups**.**

Item	Serum EPO (mU/ml) mean value ± SD (Range)	F	P- Value
Control group	8.14 ± 3.6 (3.6 – 13.6)	26.94	< 0.001 HS
Group I	19.32 ± 5.6 (12.6 – 31.2)		
Group II	26.97 ± 8.8 (14.8 – 41.3)		
Group III	31.48 ± 6.9 (18.5 – 43.2)		
Group IV	38.36 ± 14.3 (19.2 –72.6)		

Table (4): Correlation between serum EPO level (mU/ml) and some laboratory parameters**.**

Item	R	P value
HB	-0.30	0.020 S
RBC_S	-0.142	0.281 NS
WBC_S	-0.239	0.066 NS
Platelets	-0.216	0.097 NS
SGPT	0.073	0.578 NS
SGOT	0.114	0.387 NS
Total bilirubin	0.192	0.141 NS
Serum albumin	0.358	0.005 S
PT	0.073	0.582 NS
AFP	0.454	< 0.001 HS
Blood urea	0.354	0.006 S
Serum creatinine	0.218	0.101 NS
FBS	0.181	0.166 NS

Table (5) : Least significance difference (LSD) for serum EPO level (mU/ml) among the studied groups of patients and control group**.**

Group	Control group	Group I	Group II	Group III
Group I	<0.001 HS			
Group II	<0.001 HS	<0.05 S		
Group III	<0.001 HS	<0.001 HS	>0.05 NS	
Group IV	<0.001 HS	<0.001 HS	<0.001 HS	<0.05 S

Table (6): Serum EPO level among patients and control & treated and untreated patients**.**

Parameter	Serum EPO level (mU/ml)	P Value
Patients (N= 60)	24.85 ± 13.44	<0.001 HS
Control (N= 15)	8.14 ± 3.67	
Treated patients (N= 30)	34.92±11.58	<0.001 HS
Untreated patients (N= 30)	23.14 ± 8.26	

NS=Non significant. S= Significant. HS=Highly significant.

Discussion:

Anemia is a multi-factorial complication of chronic liver disease. It occurs due to impaired iron reutilization, low-grade haemolysis, shortened red blood cell life span, hypo-secretion of EPO and tissue hypo responsiveness to EPO. This is secondary to the action of inflammatory cytokines which among other effects increase the production of hepcidin, an iron regulatory hormone synthesized predominantly in the liver. Some reports have shown inadequate hepcidin expression in chronic hepatitis C facilitating the iron deposition in the liver. Portal hypertension may be associated with chronic blood loss into the gut with development of chronic iron deficiency anemia **[6,7]**.

The use of recombinant human EPO is recommended as adjuvant therapy during PEG-IFN and RBV therapy. It is a physiological endogenous erythroid growth factor, it cannot be disputed that EPO improves hemoglobin levels in the setting of HCV treatment **[8]**.

Barsoum et al. **[9]** concluded that plasma EPO level increased in patients with liver cirrhosis than normal level and was directly related to the severity of liver cirrhosis especially when associated with functional renal impairment. But, increased level of EPO cannot compensate for anemia during the progression of liver cirrhosis.

This study revealed a non-significant difference of serum EPO levels between the studied groups as regards age and gender. Similar results were obtained by Louis et al. **[10]** who found no difference in endogenous EPO-stimulated red blood cell volume expansion between males and females.

Our study reported statistically higher levels of serum EPO in patients when compared to controls and in treated patients when compared to untreated patients; it differed significantly among the studied groups of patients (P-value <0.001) (Tables 5 and 6). Mihaila and his colleagues mentioned that chronic infection with HCV is associated with the production of serum and intra-hepatic inflammatory cytokines**.** The increased release of the pro-inflammatory cytokines determines the augmentation of the EPO synthesis **[11-13]**.

Also this study revealed that, higher levels of EPO were found in anemic patients with chronic HCV infection when compared with those who were non-anemic and the controls. This was in agreement to Bruno et al. **[14]** who stated that anemic patients with chronic hepatitis C infection had higher serum EPO level than non-anemia and serum EPO level was significantly lower in healthy subjects than chronic HCV infected patients.

In this study, group IV had the highest levels of serum EPO. This goes well with the study done by Kowdley **[15]** who stated that the etiology of anaemia induced by HCV combined therapy is multifactorial. It is a "mixed anaemia" in which both haemolysis by RBV and bone marrow suppression by PEG-INF occur simultaneously and there was no correlation between serum EPO level and duration of therapy.

Interestingly, the present study didn't show significant difference in serum EPO levels between group II and group III, and showed a negative correlation between serum EPO and HB levels among the other studied parameters. In contrast, Siciliano et al. **[16]** found that EPO values were not related to the degree of both HB concentration or the severity of liver dysfunction in chronically HCV patients. However in the present study, we didn't determine its relation to the severity of hepatic dysfunction as all patients had matched Child scores. This discordance could be due to different population size and the allocation of patients in subgroups.

Considering that RBV is concentrated in erythrocytes and has a long half-life of 40 days resulting in oxidative damage to the red cell membrane and hemolysis**.** Anemia results when erythrocytes are destroyed faster than erythropoiesis can compensate **[16-18]**. Several questions can arise in the minds of clinicians when faced with potential new drugs including efficacy and the cost-benefit ratio. Medhat and Yousri **[19]** reported that pentoxifylline and vitamin E can ameliorate RBV-induced haemolysis; improve compliance and virologic clearance when combined with the standard antiviral therapy in patients with chronic hepatitis C. Also, erythropoietin, which is not approved by the U.S. Food and Drug Administration (FDA) for use in patients with HCV infection, adds another parenteral drug to the treatment regimen, and is associated with additional costs, inconvenience, and potential side effects **[20]**.

So, it is recommended to redirect the future studies of large number of patients towards other alternative adjuvant therapies for anemia associated with combined treatment with PEG-INF and RBV for HCV patients; also, we should look for other factors that may cause anemia such as patient iron profile and bone marrow capacity.

Finally, we can conclude that chronic liver disease by HCV does not affect the physiological EPO response to anemia. Serum EPO level was significantly higher in chronically infected patients with HCV compared with healthy control group. Also serum EPO level increased in anemic more than non anemic patients specially when treated with PEG-INF and RBV. Reflecting that the rationale for EPO treatment of anemia in those patients is not straight forward.

References:

1- **Jelkmann W (1992):** Erythropoietin structure, control of production, and function. Physiol Rev; 72:449–489.

2- **Weidemann A and Johnson RS (2009):** Non renal regulation of EPO synthesis. Kidney Int.; 75(7):682-8.

3- **McHutchison JG, Manns MP and Longo DL (2006):** Definition and management of anaemia in patients infected with hepatitis C virus. Liver International; (26): 389–98.

4- **Jelkmann W (1992):** Erythropoietin: structure, control of production, and function. Physiol Rev; 72:449–89.

5- **National Committee for Clinical Laboratory Standards (1998):** Document H3-A4, Wayne, PA: NCCLS.

6- **Nagashima M, Kudo M, Chung H, et al. (2006):** Regulatory failure of serum prohepcidin levels in patients with hepatitis C. Hepatol Res; 36:288–93.

7- **Rosario Gonzalez-Casas, E Anthony Jones, and Ricardo Moreno-Otero (2009):** Spectrum of anemia associated with chronic liver disease World J Gastroenterol.; 15(37): 4653-58.

8- **Yoshida EM, Santos AD, Partovi N, et al. (2006):** Erythropoietin and hepatitis C therapy: useful adjuvant therapy but remember to treat the patient and not just a number. Can J Gastroenterol, 20 (8), 519-20.

9- **Barsoum I, Zaghloul SG, EL-Hady H A et al. (2011):** Plasma erythropoietin level in patients with liver cirrhosis and its relationship to the severity of cirrhosis and renal function. African Journal of Nephrology 15:16-23.

10- **Louis D, Galpin JE, Levine JD, et al. (2005):** Recombinant human erythropoietin for patients with AIDS treated with zidovudine. N Engl J Med; 322:1488–93.

11- **Mihaila M, Romeo G and Elena C (2009):** Cytokine pattern correlate with liver damag in patients with chronic hepatitis C. Ann Cin Lab Sci; 36.144-50.

12- **Hassan M, Selimovic D, Ghozlan H et al. (2007):** Induction of high molecular weight tumor necrosis factor alpha by hepatitis C virus non structural protein 3 in liver cells in AP.1 and NF kappa B depended activation. Cell signal;19: 301-13.

13- **Maiese K, Li F and Chung ZZ. (2005):** New exploration for erythropoietin. JAMA; 293: 90-95.

14- **Bruno CM, Neri S,Sciacca C et al. (2004):** Plasma erythropoietin levels in anaemic and non-anaemic patients with chronic liver diseases. World J. Gastroenterol.; 10(9): 1353-56.

15- **Kowdley KV (2005):** Hematologic side effects of interferon and ribavirin therapy. J Clin Gastroenterol; 39 (Suppl 1):S3–8.

16- **Siciliano M, Eleonora B and Franceschelli A (2005):** Erythropoietin secretion in patients with liver cirrhosis. Scand J Gastroenterol; 40: 122-23.

17- **Glue P (1999):** The clinical pharmacology of ribavirin. Semin Liver Dis;19 Suppl 1:17-24.

18- **De Franceschi L, Fattovich G, Turrini F et al. (2000):** Hemolytic anemia induced by ribavirin therapy in patients with chronic hepatitis C virus infection: Role of membrane oxidative damage. Hepatology; 31:997-1004.

19- **Mehdat Assem and Yousri M (2012):** Impact of Pentoxifylline and Vitamin E on Ribavirin-Induced Haemolytic Anaemia in Chronic Hepatitis C Patients: An Egyptian Survey. Euroasian Journal of Hepato-Gastroenterology; 2(1):35-40 .

20- **McHutchison JG, Manns MP, Brown RS Jr, et al. (2007):** Strategies for managing anemia in hepatitis C patients undergoing antiviral therapy. Am J Gastroenterol.; 102: 880-89.