Study of plasma erythropoietin hormone level in patients with liver cirrhosis

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Abstract: Background and aims: Circulating erythropoietin (Epo) levels were found to be increased in patients with acute and chronic liver diseases. This may be due to impaired liver function and its possible influence on Epo catabolism, inflammation, through the liberation of cytokines with a modulating action on Epo**.** The aim of the present study was to evaluate the level of plasma erythropoietin hormone level and its relation to anemia in cirrhotic patients. **Methods:** 60 cirrhotic patients and 20 healthy persons of matched age and sex as a control group were selected. All were subjected to full clinical; laboratory and ultrasonographic assessment to diagnose liver cirrhosis and accordingly, cirrhotic patients were classified into 3 groups (G1a: Child's grade A; G1b: Child's grade B; G1c: Child's grade C) 20 patient in each group. Plasma Epo was detected to all subjects. **Results:** High significant elevation of mean plasma Epo values as well as Epo Hb normalized ratio in cirrhotic groups (G1a, G1b & G1c) when compared to control group (G2) $p<0.001$. Significant elevation in mean plasma Epo value of anemic patients when compared to non-anemic patients $p<0.05$. Highly significant negative correlation between mean plasma Epo value and hemoglobin (Hb) concentration $p<0.01$. **Conclusion**: Plasma Epo levels are significantly higher in patients with liver cirrhosis, plasma Epo levels in anemic cirrhotic patients are significantly higher than that of non-anemic cirrhotic patients and Hb concentration is not the only factor responsible for elevated plasma Epo level in cirrhotic patients.

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1. Introduction:

Erythropoietin (Epo) is a low-molecular-weight glycoprotein hormone stimulator of erythropoiesis produced in the fetal liver and subsequently in the adult kidney. Epo is a pleiotropic cytokine that exerts diverse biological effects in many non-haematopoietic tissues [1]. There is increasing evidence suggesting a wider biological role for Epo/EpoR unrelated to erythropoiesis. Circulating Epo levels were found to be increased in patients with acute and chronic liver diseases. This may be due to impaired liver function and its possible influence on Epo catabolism, inflammation, through the liberation of cytokines with a modulating action on Epo, and direct Epo production by the liver cells [2]**.**

Tacke et al., [3] reported that plasma Epo levels were significantly elevated in chronic liver disease patients, and that Epo increased according to child's stage of cirrhosis, independently of the cause of cirrhosis.

Chronic anemia is frequently observed in patients with liver cirrhosis and is one of the factors predicting survival in patients with advanced hepatic diseases [4].

Some studies have reported that liver cirrhosis is accompanied by an upregulation of EPO levels in response to anemia, bleeding complications, impaired pulmonary function, thrombocytopenia and liver dysfunction[5]. However, it is unclear whether the low hemoglobin is the only relevant factor for EPO regulation in liver diseases. IL-6 dependent pathways could be an additional factor mediating the EPO upregulation [3].

Hepatic EPO production increases under conditions of lowered oxygen supply (6). Apart from the effect of hypoxia, several agents may modulate EPO production in human hepatoma cultures similar to their effects in vivo [7]. The EPO synthesis-increasing factors include Interleukin - 6 (IL-6), thyroid hormones, and vitamin A, while inhibition is exerted by the pro-inflammatory cytokines Interleukin – 1(IL-1) and tumor necrosis factor-alpha (TNF-α) as well as by reactive oxygen species [8].

2. Patients and methods:

2.1. Patients

The current study was case control study conducted to a total of 60 cirrhotic patients were selected from the Outpatient Clinic and/or Inpatient Department of Tropical Medicine and National Liver Institute, Menoufiya University in the period between December 2007 to April 2008. They were 33 (55%) males and 27 (45%) females and their ages were ranging from 37 to 66 years with a mean value of (54.55± 7.2), as well as 20 healthy persons of matched age and sex as a control group. Patients with Organic renal disease; blood diseases; Recent history (6 months) of bleeding, blood transfusion, iron or Epo supplementation ; splenectomy, shunt operations, decongestion and TIPS; Any malignancy including hepatocellular carcinoma (HCC) were excluded from the current study.

2.2. Clinical and biochemical assessment of the patients:

All patients were subjected to full history taking including history of alcohol or drug abuse, thorough clinical examination and laboratory investigations in the form of: complete blood count, conventional liver biochemical tests [aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, serum albumin, prothrombin time and concentration]. Abdominal ultrasound was done for all patients. Diagnosis of cirrhosis was done by clinical examination, ultrasonographic findings and laboratory investigations.

2.3.classificaions of the patients

Group 1: comprised (60) cirrhotic patients, they were subdivided into 3 subgroups according to Child-Pugh classification:

- Group 1a: comprised (20) cirrhotic patients (Child's grade A)
- Group 1b: comprised (20) cirrhotic patients (Child's grade B)
- Group 1c: comprised (20) cirrhotic patients (Child's grade C)
- Group 2: comprised (20) healthy controls

2.4.Assessment of plasma erythropoietin hormone level (by ELISA technique):

2 ml blood sample was withdrawn from every subject in the current study. Grossly hemolytic samples were discarded. Samples were stored immediately at a temp of -20 Celsius. In this sandwich type assay erythropoietin is first bound by a rabbit anti-EPO- antiserum immobilized on the solid phase of a micro-titer plate. After a washing step a second antibody to erythropoietin from rabbit conjugated to biotin forms a sandwich complex with EPO. After another incubation with an anti-biotin antibody conjugated to alkaline phosphatase and addition of a substrate (PNPP), a yellow color is formed which is proportional to the concentration of erythropoietin [9].

2.5.Statistical Methods:

Data were expressed as mean ± SD and percentages. Mean values between different groups were compared using one way ANOVA test and post Hoc test. Correlations were performed with Pearson standard linear regression analysis. The SPSS package for windows was used for the analysis. $p \leq 0.05$ was considered significant, $p < 0.001$ was considered highly significant and $p > 0.05$ was considered insignificant .

3. Results: Demographic data and causes of liver cirrhosis were represented in table 1**.** The mean values of Hb concentration and platelet count in cirrhotic groups (G1a, G1b & G1c) were significantly lower than that of G2.The mean values of Hb concentration in G1b & G1c were significantly lower than that of G1a, and that of G1c was significantly lower than that in G1b ($p<0.001$). The mean values of platelet count of G1b & G1c were significantly lower than that of G1a ($p<0.001$). On the other hand, there was no significant difference between the studied groups as regards mean total leucocytic count as shown in table 2. High significant elevation of mean plasma Epo values in cirrhotic groups (G1a, G1b & G1c) when compared to control group ($f=69.93$, $p<0.01$, sig.).While there was no significant difference between G1a, G1b & G1c as regards mean plasma Epo values. Mean values of Epo-Hb normalized ratio were significantly high among cirrhotic when compared to controls ($f=41.7$, $p<0.001$, sig.)as shown in table3. There was highly significant negative correlation between mean Hb concentration and mean plasma Epo values in all groups ($r=-0.668$, $p<0.01$, sig.). There was highly significant negative correlation between mean plasma Epo values as regards platelet count; serum albumin and prothrombin concentration. Statistical analysis revealed significant negative correlation between Epo-Hb normalized ratio as regards platelet count; serum albumin and prothrombin concentration as shown in table 4. There was significant elevation in mean plasma Epo value of anemic patients when compared to non-anemic patients ($t=2.278$, $p<0.05$, sig.) as shown in table 5.

Table 1: general characteristics of studied groups of patients and controls.

	Group 1a N= 20	Group 1b N= 20	Group 1c N= 20	Group 2 N= 20	P value
Age (Mean ±SD)	51± 7.22	55.15 ± 7.26	56.65 ± 6.66	53.35 ± 6.84	NS
Sex Male no (%) Female no (%)	 11(55) 9(45)	 12 (60) 8(40)	 10(50) 10(50)	 11(55) 9(45)	NS
Cause of liver cirrhosis: HCV no (%) HBV no (%) Schistosomiasis no (%)	 18 (90) 2(10) 8(40)	 17(85) 2(10) I3(65)	 15(75) 4(20) 8(40)	 0(0) 0(0) 0(0)	NS

Table 2: laboratory investigations for studied groups of patients and controls.

	Group 1a N= 20	Group 1b N= 20	Group 1c N= 20	Group 2 N= 20	P value
CBC: Hb :13-18 gm/dl Mean ±SD	 12.63 ± 1.5	 10.84 ± 2.46	 9.81± 1.35	 14.02 ± 0.66	 <0.001
TLC :4.3-10.8 × 10^3/mm^3 (Mean ±SD)	 7.46 ± 3.22	 7.02 ± 3.16	 6.81± 4.84	 5.37 ± 0.86	0.23 <0.001
PLT : 150,000 - 350,000/mL Mean ±SD	 210.35± 89.54	 129 ± 52.97	 125.1 ± 56.01	 273.85 ± 56.58	
Liver function tests:					
ALT: 1 - 21 units/L Mean ±SD	 41.3 ± 35.6	 49.9 ± 50.64	 47.6 ± 23.66	 15.15 ± 2.79	<0.005
AST : 7 – 27 units/L Mean ±SD	 54.2 ± 41.6	 59.4 ± 32.35	 76.1 ± 44.08	 24.4 ± 3.16	<0.001
T.Bilirubin : up to 1.0 mg/dL (Mean ±SD)	 0.98 ± 0.38	 2.47 ± 4.46	 4.02 ± 3.45	 0.53 ± 0.23	 <0.001
S.Albumin: 3.5 - 5.0 gm/dL (Mean ±SD)	 3.5 ± 0.38	 2.74 ± 0.29	 2.35 ± 0.24	 4.02 ± 0.23	 <0.001
Prothrombin concentration (%) Mean ±SD	 80.5 ± 9.98	 66.8 ± 11.03	 50.4 ± 12.05	 97.5 ± 1.38	 <0.001

Table 3: mean values of Epo and Epo-Hb normalized ratio

	Group 1a N= 20	Group 1b N= 20	Group 1c N= 20	Group 2 N= 20	P value
Epo: 0-19 mIU/mL Mean ±SD	51.05 ± 11.79	55.05 ± 14.26	56.55 ± 16.35	8.13 ± 1.9	<0.001
Epo-Hb normalized ratio Mean ±SD	4.15 ± 1.32	5.44 ± 2.1	6 ± 2.29	0.58 ± 0.14	<0.001

Table 4: Correlation coefficient of Epo and Epo-Hb normalized ratio to hemoglobin; platelet count; serum albumin and prothrombin concentration.

Variables	Epo		Epo-Hb normalized ratio	
	r	p value	r	p value
Hemoglobin	-0.668	<0.01		
Platelet count	-0.634	<0.01	-0.73	<0.01
Serum albumin	-0.723	<0.01	-0.76	<0.01
prothrombin concentration	-0.724	<0.01	-0.79	<0.01

Table 5: comparison between anemic and nonanemic patients as regard mean values of Epo.

	Anemic patients (n= 37)	Non anemic patients (n= 23)	t	P value
Epo mIU/mL (Mean ± SD)	57.2 ± 14.89	49.3 ± 11.76	2.278	<0.05

4. **Discussion**

Researches about circulating Epo in patients with liver diseases were few and results were contradictory [10].

.In the present study, highly significant elevation of mean plasma Epo values was seen in cirrhotic groups (G1a, G1b & G1c) when compared to control group (G2). This result is in agreement with *Yang et al.,* [11] who proved that mean plasma Epo values were significantly higher in cirrhotic patients than that in healthy controls. Similar results were found by *Tacke et al* who concluded that mean plasma Epo values were higher in chronic liver disease patients than that of control[3]. Also, mean plasma Epo values were higher in cirrhotic patients than that of non-cirrhotic patients. On the other hand, Siciliano *et al*. demonstrated a reduced plasma level of EPO in patients with cirrhosis compared with non-cirrhotic patients [12]. Several factors may contribute to these discrepant results. In the control group of the study of Siciliano *et al*., 23 out of 34 subjects had iron-deficiency anemia, while only 11 were healthy subjects. Furthermore, the plasma EPO levels were expressed as logarithmic values. Thus, the difference between plasma EPO levels from cirrhotic patients and healthy subjects may be masked by these factors [13].

Theoretically, the increase in plasma EPO levels may be the result of either an increase in production or a decrease in catabolism. Jensen *et al*. have demonstrated that a normal metabolism of EPO was maintained in patients with liver cirrhosis [14]. Accordingly, increased production rather than decreased catabolism of EPO may be the important factor that determines the increase in plasma EPO levels in cirrhotic patients. It is very important to understand alteration of cytokines and different growth factors associated with pathogenesis of liver cirrhosis and have an effect on upregulation of Epo [11].

In the present study, HCV infection was present in the majority of cirrhotic groups. Some reports demonstrated higher Hb and HCT levels in Hepatitis C virus (HCV)-positive haemodialysis (HD) patients compared to HCV-negative patients [15]. This was also concluded from the recent Egyptian study in which Sabry et al referred these findings to increased production of EPO from HCV-infected patient's liver [16].

Chronic anemia is a common clinical complication in patients with liver cirrhosis and is regarded as an important prognostic factor [17]. It is also known that the liver can produce as much EPO as the kidney normally produces in the state of anemia. [18]. Consistent with previous studies, the present study also demonstrated that cirrhotic patients with anemia had higher plasma levels of EPO than those without anemia

We also demonstrated a highly significant negative correlation between mean Hb concentration and mean plasma Epo values in all groups**.** This was in agreement with the findings of *Tacke et al.,* [3] who showed a highly significant negative correlation between mean plasma Epo and mean Hb concentration.

This inverse correlation between Epo and hemoglobin suggests that the physiological Epo response to anemia is preserved in cirrhotic patients, although this rise in Epo level is not sufficient to improve anemic state of these patients. This might be due to the ongoing blood loss, hypersplenism, malabsorpotion, malnutrition, infection and diarrhea [19]. Moreover, Bruno *et al.* reported that increased EPO plasma levels were only detected in cirrhotic patients with hemoglobin concentrations below 12 g/dL [13]. They suggested that inflammatory cytokines, namely interleukin-1, tumor necrosis factor and transforming growth factor, are enhanced in liver diseases and have been found to inhibit hypoxia-induced erythropoietin production *in vitro* and *in vivo*[20-22]

In our study, in order to analyze whether the high Epo levels in cirrhotic was not only due to anemia; but also due to liver dysfunction, we eliminated the effect of anemia on Epo values by normalizing Epo values to Hb concentration. Our results revealed that Epo-Hb normalized ratio was significantly higher in cirrhotic patients than that of control group. Furthermore, Epo-Hb normalized ratio significantly increased with Child-Pugh class. Epo-Hb normalized ratio showed a significant negative correlation with platelet count, serum albumin, and prothrombin concentration.

The present study revealed highly significant negative correlation between mean plasma Epo values and serum albumin level and prothrombin concentration in all groups. This was consistent with the findings of Tacke et al., [3] and Yang et al., [11] who stated that Epo inversely correlated with albumin level and prothrombin concentration**.**

Conclusion:

Plasma Epo levels are significantly higher in patients with liver cirrhosis, plasma Epo levels in anemic cirrhotic patients are significantly higher than that of non- anemic cirrhotic patients and Hb concentration is not the only factor responsible for elevated plasma Epo level in cirrhotic patients.

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