Research Article

Evaluation of Serum Erythropoietin Level in Patients with Chronic Hepatitis C under Antiviral Therapy

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Abstract

Background: Interferon (INF)-Ribavirin therapy for patients with chronic viral C hepatitis, is associated with the development of anemia which affects treatment efficacy and tolerability. Hemoglobin (Hb) concentrations decrease and Serum erythropoietin (EPO) levels increase during the first weeks of treatment. **Aim of the study:** To evaluate the serum EPO level in patients with chronic HCV before and after treatment. **Patients and methods:** Forty patients with chronic hepatitis C infection were included in this study. They were scheduled for treatment with Pegylated Interferon (Peg- INF) and Ribavirin for ٤٨ weeks. Serum EPO was measured before and after INF–Ribavirin treatment. Statistical analysis was applied to compare the level of EPO before and after therapy to estimate the endogenous excess EPO production in response to treatment induced anemia. **Results:** EPO concentration increased significantly in HCV patients during the first weeks of treatment from ١٩.٤٠±١٢.٨ to ٤٠.٤٧±١٠.٩ (p=٠.٠٠٠١) however Hb concentrations decreased from ١٤.٣٤±١.٢g/dl to١١.١٠±١.٣g/dl (P=٠.٠٠٠١). **Conclusion:** Anemia is a common adverse effect during INF- Ribavirn therapy for chronic hepatitis C. The reduction of the Hb level is physiologically accompanied by an increase in the serum EPO level.

Key words: Chronic hepatitis C- Anemia – Erythropoietin and HCV therapy.

Introduction

Hepatitis C infection is one of the most prevalent causes of chronic liver disease with an estimated ٢١٠ million people chronically infected worldwide (Averhoff F. M., et al., ٢٠١٢). Combined treatment increases the efficacy of therapy, i.e. it increases the rate of sustained viral response (SVR) (Jacobson I.M., et al., ٢٠٠٧).

Anemia secondary to chronic viral hepatitis C has a complex etiology, including deficient iron use, an increased degree of hemolysis and decreased life duration of erythrocytes, low erythropoietin secretion, along with a reduced tissue response to erythropoietin (Ganz T. ٢٠٠٤).

INF-Ribavirin treatment has numerous adverse effects; it causes anemia, neutropenia, and thrombocytopenia in particular. Anemia is associated with a decrease in the quality of life, with the need for the reduction of Ribavirin doses or even the cessation of treatment, which most frequently results in a weaker response to treatment (Chao-Hung H., et al., ٢٠٠٦).

Anemia in such treated patients has several causes: hemolytic anemia, pernicious anemia and aplastic anemia, secondary to treatment and nutritional deficits present in variable degrees in all

chronic diseases. About ٥٠٪ of patients who receive combined Pegylated Interferon alpha ٢a and Ribavirin therapy develop anemia and dose reduction mostly is required in about one fifth of patients (McHutchinson J.G., et al, ٢٠٠٦).

Some studies suggest that high serum Ribavirin levels cause an increase in the SVR rate. However, the increase of Ribavirin doses should also take into account the adverse effects of therapy. The low Hb level at the onset of therapy and the high serum Ribavirin level are predictors of anemia in patients treated with Ribavirin (Lindalh K., et al., ٢٠٠٥). About one fifth of the patients required the adjustment of Ribavirin doses due to anemia (Fried M.W., et al., ٢٠٠٢).

Ribavirin enters the erythrocytes with the help of a nucleoside transporter and is initially converted to Ribavirin monophosphate, subsequently to riba-virin diphosphate and triphosphate. The accumulation of Ribavirin phosphates along with the relative adenosine triphosphate deficiency increases the susceptibility to oxidative processes, causing an increase in cellular toxicity and subsequently extravascular hemolysis (McHutchinson J.G., et al., ٢٠٠٦)

EPO is an endogenous hormone that causes an increase in the number of progenitor cells of the erythrocytic series in the blood-forming marrow (Balan V., et al., ٢٠٠٥). Its levels are regulated by blood oxygen content and several other factors interacting in a complex manner (Bisceglie A.M., et al., ١٩٩٤). When oxygen levels fall, as in anemia, and renal hypoxia, EPO blood level increases and stimulates red blood cell volume reconstitution (Bruno C.M., et al., ٢٠٠٧). Serum EPO levels increased during the first ٨ weeks of treatment in all the patients who completed the therapy without requiring ribavirin dose reduction (Balan V., et al., ٢٠٠٠)

Patients and methods

This prospective interventional study was undertaken at the virology clinics of Minia University Hospital, Minia, Egypt from January ٢٠١٢ to January ٢٠١٣. Forty Patients with histologically confirmed chronic hepatitis C by liver biopsy and who fulfilled the criteria for treatment were enrolled in this study. All patients were treated weekly with ١٨٠ mg subcutaneous peginterferon alpha-٢a and oral ribavirin at a total daily dose of ١٠٠٠–١٤٠٠ mg according to body weight. All patients were subjected to the following:-

I- Complete history taking and thorough clinical examination.

II- Laboratory investigations:

١- HCV Ab was carried out using fully automated ELISA (Axsym).

٢- Quantitative HCV RNA level by PCR: for estimation of viral load, the test was carried out using the real time PCR system. Quantitative HCV PCR was done before treatment and after ١٢ weeks of treatment. Therapy was continued only for responders and those with more than ٢ log decrease in viral load after ١٢ weeks of treatment and done after ٢٤ weeks of treatment and therapy was continued only if PCR became negative.

٣- Liver functions and renal functions: using the fully automated clinical chemistry Kone labe finloud.

٤- Prothrombin time using Kits supplied from Simens Company.

٥- Complete blood count (CBC) was carried out using Minidry ٣٢٠٠ auto cell counter.

٦- Serum EPO concentrations were determined using Kits supplied from DRG Company.

٧-Thyroid profile: as a base line, as autoimmune thyroiditis can be caused or exacerbated by pegylated interferon. It was carried out using fully automated ELISA (Axsym).

III- Abdominal ultrasonography: to evaluate liver size and parenchyma and exclude ascites.

IV- Liver biopsy: to determine the grade of inflammation and the stage of fibrosis, Liver biopsy results and the METAVIR score were recorded for every patient. We included patients with minimal changes in the liver biopsy with Metavir score >A١ and >F٠ with elevated liver enzymes and Those with normal liver enzymes and Metavir score ≥ A٢ and ≥ F٢.

V- Upper GIT endoscopy: to exclude esophageal varices.

VI- Fundus examination.

VII- Electrocardiography (ECG).

Patients with renal failure, hepatic failure, cardiac failure, ischemic heart diseases, major neurological & psychiatric disorder and those with hepatitis B surface antigen positive test ,body mass index (BMI) >٣٥, pregnant & lactating women were excluded from the study. Also we excluded HCV- patients who have platelets count <٨٠.٠٠٠ and white blood cells (WBCs) count <٢٥٠٠ & Hb <١٢g/dl.

Ethical approval of the study

Ethical approval for the study was obtained from EL-Minia university Ethics committee in accordance with the١٩٧٥ Declaration of Helsinki. Also informed consent was obtained from patients before starting the study

Statistical analysis

Data entry and analysis were all done with an IBM compatible computer using SPSS software version ١٣ (SPSS, Chicago, IL, USA).Results were expressed as mean ± standard deviation (SD), or number (%). Comparisons between the means were done using the paired t-test. Comparisons between categorical data (n (%)) were done using the Chi-square test. Correlation was used to describe the strength and direction of the linear relationship between two variables. A p-value of less than ٠.٠٥ was considered significant; <٠.٠١ was considered highly significant.

Results

This study included forty patients with chronic HCV infection, scheduled for treatment with Peg- INF and ribavirin therapy for ٤٨ weeks. Eight of them were excluded by ٢٤th week, being have positive PCR test for HCV RNA according to the protocol of therapy. The demographic data are shown in (Table ١). The Hb level was significantly reduced in patients after the therapy (p=٠.٠٠٠١) (Table ٢). Anemia was a prominent adverse effect developed with treatment in approximately ٨١.٢٥٪ of cases (Table٣). The mean EPO level was significantly increased after treatment in patients developing anemia (p=٠.٠٠٠١) (Table ٤). There was a significant fair correlation between Ribavirn doses and EPO level (p=٠.٠٢) (Fig ١). There was a weak correlation between INF doses and EPO level (p=٠.٢) (Fig ٢).Also, there was a strong correlation between duration of treatment and the increase in serum EPO level (p=٠.٠٠٠١) (Fig ٣).

Table ١: Demographic data of the patients included in the study

	The studied group (٤٠ patients)
Age (Mean ±SD)	٥٠.٨±١٤.٤
Gender **Male** **Female**	 ٢٨ (٧٠٪) ١٢ (٣٠٪)
Smoking **Smokers** **Non smokers**	 ١٦ (٤٠٪) ٢٤ (٦٠٪)

Residence	
Rural	٣١(٧٧.٥٪)
Urban	١٩(٢٢.٥٪)

Table (٢): Haemoglobin level in patients before and after treatment.

Hematological Parameters	Before treatment (Mean±SD)	After treatment (Mean±SD)	P-value
Haemoglobin	١٤.٣٤±١.٢g/dl	١١.١٠±١.٣g/dl	٠.٠٠٠١

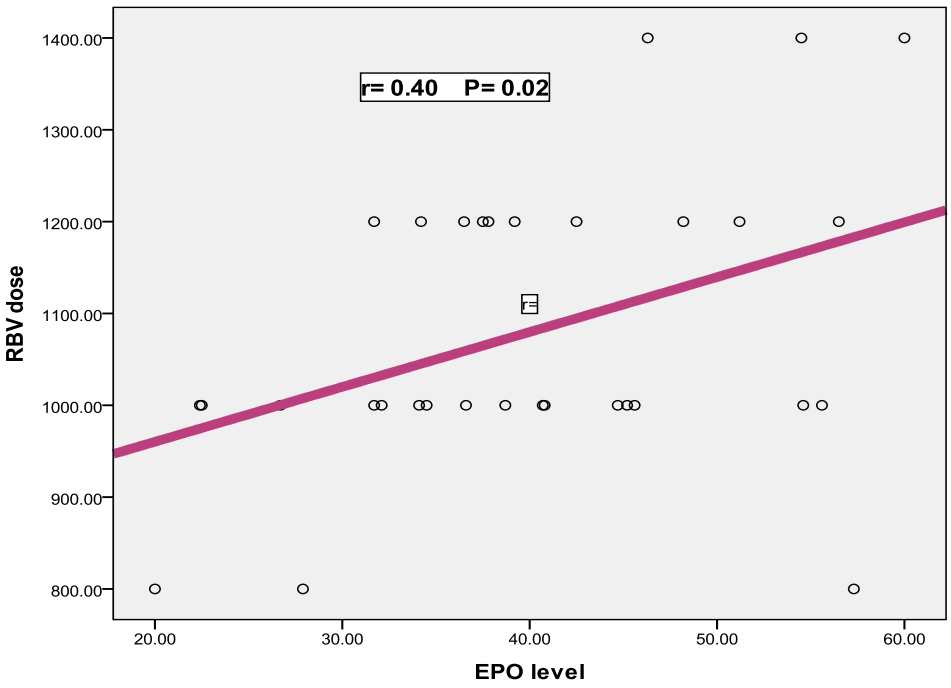
Table (٣): Percentage of anemia in HCV patients before and after treatment.

Anemia	Cases			
	Before treatment		After treatment	
	N	%	N	%
Anemic	٠	٠	٢٦	٨١.٢٥
Non-anemic	٤٠	١٠٠	٦	١٨.٧٥
Total	٤٠	١٠٠	٣٢	١٠٠

Table (٤): Serum EPO level in patients developing anemia with treatment.

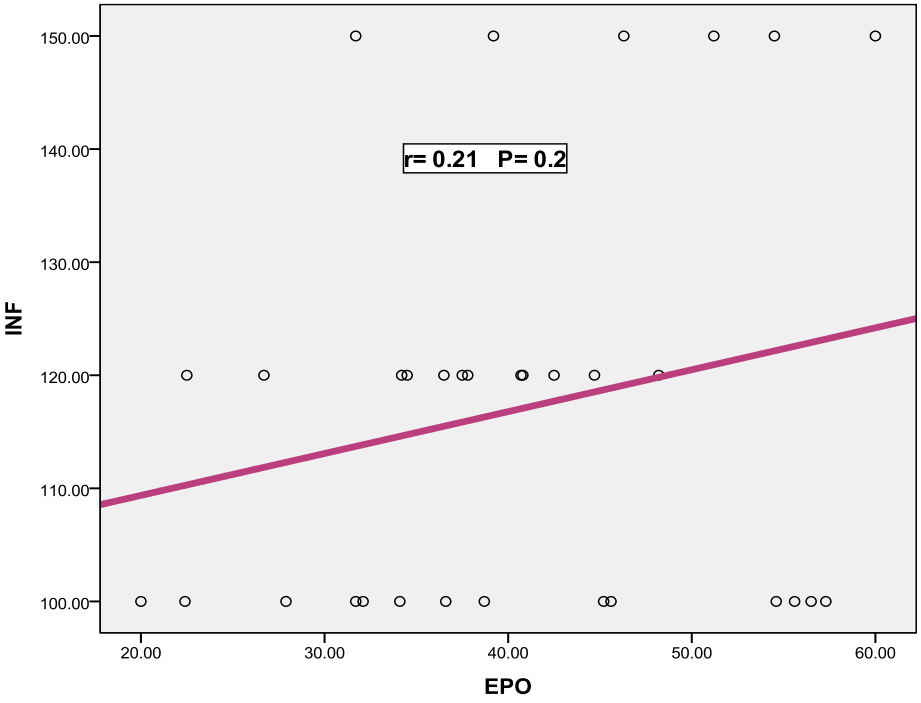
Mean EPO level	Patients developing anemia (Mean±SD)	P-value
Before treatment	١٩.٤٠±١٢.٨	٠.٠٠٠١
After treatment	٤٠.٤٧±١٠.٩	

Figure ١: The relation between Ribavirin (RBV) doses and serum EPO level.



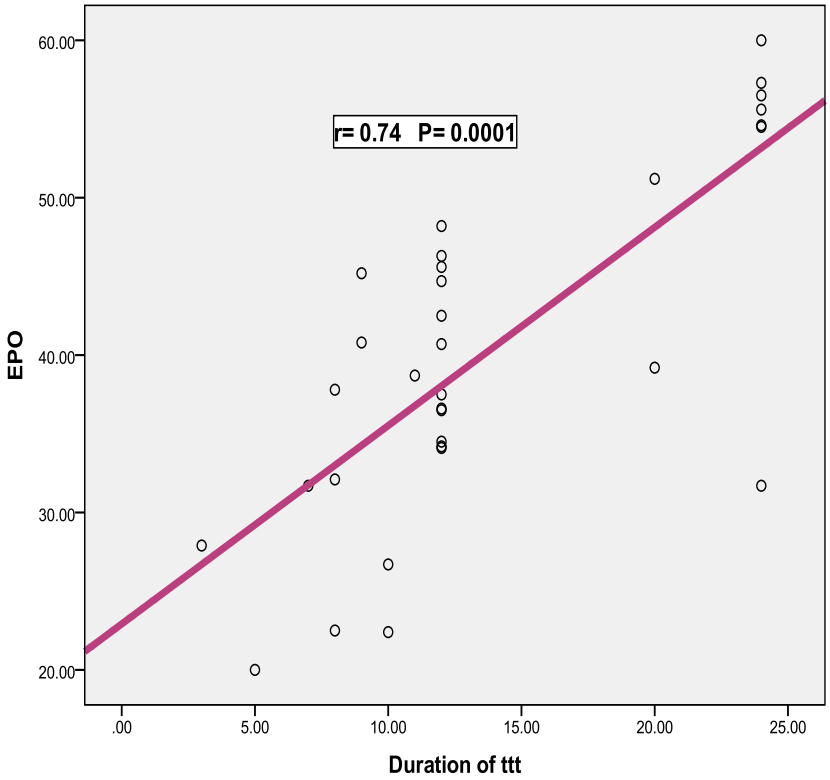
٠-٠.٢٤ no or weak ٠.٢٥-٠.٤٩ fair ٠.٥٠-٠.٧٤ moderate ٠.٧٥ or more strong

Figure ٢: Correlation between peg Interferon doses and the serum EPO level.



٠-٠.٢٤ no or weak ٠.٢٥-٠.٤٩ fair ٠.٥٠-٠.٧٤ moderate ٠.٧٥ or more strong

Figure ٣: Correlation between of duration of treatment and serum EPO level.



٠-٠.٢٤ no or weak ٠.٢٥-٠.٤٩ fair ٠٥٠-٠.٧٤ moderate ٠.٧٥ or more strong

Discussion

In Egypt, HCV infection reached an epidemic level. However, combination antiviral therapy (Peg INF and Ribvirin) allowed many patients infected with HCV to achieve a SVR (Sabry AA, ٢٠٠٢, Sherman M, ٢٠٠٤).

Anemia, leucopenia and thrombocytopenia are among the numerous side effects of antiviral treatment. Ribavirin mainly causes anemia by extravascular hemolysis and suppression of erythropoiesis by downregulation of EPO receptors while INF causes anemia by suppression of hematopoeitic progenitor cell proliferation. (De Franceschi L, et al, ٢٠٠٠)

In this study it was found that anemia is a very common adverse effect in HCV patients receiving antiviral therapy (٨١.٢٥٪). Van Vlerken LG, et al, (٢٠١٠), studied ١٥٥ chronic hepatitis C patients on Peg -INF-Ribavirin treatment and concluded that about ٩٩٪ of patients developed anemia. However, De franceschi L, etal, (٢٠٠٠), suggested that one of the proninant adverse effects is anemia, occurring in approximately ٧٥٪ of patients having treatment.

With antiviral therapy, there was significant decrease in Hb level (from ١٤.٣٤±١.٢g/dl to ١١.١٠±١.٣g/dl), however, EPO level significanty increased (from ١٩.٤٠±١٢.٨ to ٤٠.٤٧±١٠.٩). These results are in agreement with a study by Bruno CM, et al, (٢٠٠٧), who studied ٢١ patients and reported significant decrease in Hb level, however EPO level shows significant increase.

In the present study it was found that serum EPO level increased significantly during antiviral therapy in patients developing anemia (from ١٩.٤٠±١٢.٨ to

٤٠.٤٧±١٠.٩). These results are in agreement with that reported by Van Vlerken, et al, (٢٠١٠), who studied ١٥٥ chronic hepatitis C patients on Peg-INF–Ribavirin treatment, and found that serum EPO level increased during the therapy with maximal decrease of Hb level. The same finding was stated by Hoda A.E, et al., ٢٠١٤, who found that patients with chronic HCV infection developing anemia with treatment had a significant higher serum EPO level.

The present study revealed that there was a weak correlation between INF doses and EPO level. This is matching with that of a study by Schmid M, et al, (٢٠٠٥), who evaluated ١٣٣ patients receiving antiviral treatment for HCV with Peg-IFN α in combination with Ribavirin, and found that serum EPO concentration increased with higher doses of IFN.

There was a fair correlation between serum EPO level and Ribavirin dose. This is in agreement with a study by Hanneke Van Soest, et al, (٢٠٠٩), who found that serum EPO level increased by increasing the Ribavirin dose.

In this study there was a fair correlation between serum EPO level and duration of treatment. This is in agreement with a study by Hanneke Van Soest, et al., (٢٠٠٩), who studied ٤٤ chronic HCV patients receiving Peg-INF& RBV treatment. The authors suggested that serum EPO increased during treatment with negative correlation with Hb levels at week ١٢. Also these results are in agreement with a study by Van Vlerken, et al., ٢٠١٠ who studied ١٥٥ chronic hepatitis C patients on Peg-INF/Ribavirin treatment and stated that serum EPO level increases during therapy.

Ahmed Amanzada, et al., (٢٠١٤) studied the reversed relation between Hb level and EPO level and suggested that these results are due to the presence of two genotypes; one is promoter and the other is suppressor.

Conclusion

Anemia is a common adverse reaction of Peg-IFN and Ribavirin treatment in patients with chronic viral hepatitis C.
The probability of anemia increases depending on the Ribavirin dose and duration of INF/Ribavirin therapy.

Recommendation

Giving recombinant EPO is essential to overcome anemia induced in such patients.

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