AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Hematological Side effects of Anti-viral Therapy in Egyptian Patients with Chronic Hepatitis C Virus

Hanan Abdel Hafez[1*], Mohamed El Beshlawy[1], Mahasen Mabrouk[1], Wafaa El-Akel[1], Tahany Awad[1,2], Naglaa Zayed[1], Gamal Esmat[1].

1Endemic Medicine and Hepatology Department, Faculty of Medicine, Cairo University, Cairo, Egypt.

2.Cochrane Hepato-Biliary Group, Copenhagen Trial Unit, Rigshospitalet, Copenhagen, Denmark

ABSTRACT

Haematological complications (neutropenia, thrombocytopenia and anaemia) of pegylated interferon/ribavirin(PEG-IFN/RBV) in chronic hepatitis C can result in treatment cessation and suboptimal results. To investigate the nature, frequency of haematological side effects in the treatment of chronic hepatitis C infection and their impact on the response to PEG-IFN/RBV therapy. This cross sectional study of 3719 Egyptian patients with chronic HCV (genotype 4), who were received PEG-IFN/RBV therapy at Cairo-Fatemic Hospital, Egypt in the context of the national program was retrieved. Patients were defined as having hematological abnormalities if they had the presence of either/or a combination of following hematological parameters at least once during follow up period; drop in hemoglobin(Hb) level <10gm/dl, absolute neutrophil count(ANC)<750 cells/mm3, and platelet(PLT)count<80 x 10^3/µL. At week 48, end of treatment response (ETR) was 61.6% and at week 72 the estimated sustained virological response (SVR) was 52.5%. Pre-treatment, Hb, white blood cell (WBCs)count, ANC, and PLT count; were not statistically related to the treatment response. However lower count of all parameters were associated with moderate or advanced hepatic fibrosis stages according to the METAVIR scoring ($p<0.001$). During anti-viral therapy, anaemia occurred in 31.8%, neutropenia in 22.3% while thrombocytopenia occurred in 10.5%. Univariate logistic regression analysis showed that drop in Hb, TLC and PLT count were significantly associated with response to treatment; p value < 0.01, 0.03,0.03 respectively. Baseline hematological parameters were not predictors of treatment response in chronic HCV (genotype 4); however; low Hb level, TLC, ANC, and PLT count were associated with advanced stages of hepatic fibrosis. Drop of Hb,TLC and PLT count during anti-viral therapy were significantly associated with response rates regardless to the type of PEG-IFN.

Keywords: HCV; IFN; Ribavirin, Haemtological, Anaemia, platelet and neutopenia.

*Corresponding Author Email: hananahh@hotmail.com

Received 23 October 2013, Accepted 03 November 2013

Please cite this article in press as: Abdel Hafez H. *et al.*, Hematological Side effects of Anti-viral Therapy in Egyptian Patients with Chronic Hepatitis C Virus. American Journal of PharmTech Research 2014.

INTRODUCTION

Chronic hepatitis C is a major cause of cirrhosis, end stage liver disease and hepatocellular carcinoma[1]. The population of Egypt has a heavy burden with HCV (14.7%)[2]**.** Ribavirin in combination with pegylated interferon alpha is the current treatment for chronic hepatitis C (HCV). Ribavirin presents a wide inter-individual pharmacokinetic variability and adequate exposure seems crucial for achieving sustained virologic response. Severe anaemia frequently occurred under ribavirin treatment and is a dose-dependent limiting side effect[3]. Unfortunately, both drugs have significnt haematological toxic effects[4]. IFN exerts anti-prolifirative effects on many cell types. So account for several undesirable effects, such as thrombocytopenia and leukopenia and can interfere with the successful treatment[5] . IFN induced thrombocytopenia and leukopenia is common, where as anaemia is more a sequel of combination therapy with ribavirin[6]. The mechanism is suppression of the bone marrow and reversible heamolysis which is a major side effect[7].The adenosine triphosphate (ATP) levels were significantly decreased in the presence of RBV and the hexosemonophosphate shunt (HMS) was increased, suggesting the presence of red cell susceptibility to oxidation and heamolysis[8]**.**

Ribavirin also induced anaemia through RBCs hemolysis so that EASL 2012 recommended and support ribavirin dose reduction for primary anaemia management. This is of particular importance for countries were no health insurance coverage for erythropoietin therapy exists during HCV therapy. Noteworthy, safety profiles were similar regardless of anaemia management strategy[9]**.**

So we aimed to investigate the nature, frequency of haematological side effects in the treatment of chronic hepatitis C infection and their impact on the response to PEG-IFN/RBV therapy.

MATERIALS AND METHOD

Study population

This cross sectional study was conducted on data belonging to 3719 adult patients with chronic HCV infection (genotype 4), of both sexes who were diagnosed by anti-HCV antibodies, HCV-RNA (Cobas Amplicor HCV Monitor v 2.0, Roche Diagnostic systems, CA), genetic study in addition to the histological evidence of chronic hepatitis C (>F1 stage of fibrosis according to the METAVIR score)[11]. Patients had been treated with Peg-IFN alpha 2a or 2b plus weight-based RBV (13-15mg/kg) from 2008-2011 at Cairo-Fatemic Hospital, in the context of the national program for the control of viral hepatitis, Ministry of Health and Population(MOHP),Cairo, Egypt. The study was approved by the ethical committee of the MOHP and all patients were

consented for the blood sampling and possible data application in future research.

Inclusion and exclusion criteria were based on the national guidelines for the treatment of HCV. Laboratory parameters included hematological tests; hemoglobin ≥ 11g/dl, total leucocytic count(TLC) ≥ 3000/mm3, absolute neutrophil count (ANC) ≥ 1500/mm^3, platelets count ≥ 100 x 10^3/µL. ; biochemical liver profile(elevated aminotransferases, normal serum bilirubin, normal serum albumin, prothrombin time>60%) in addition to normal serum creatinine, normal TSH, alpha-fetoprotein ≤ 3 times the normal and anti-nuclear antibodies < 1/80. Patients with normal aminotransferase levels were included in the treatment program only if they had at least stage 2 hepatic fibrosis. Patients with decompensated liver disease, co-infected patients with HBV (positive HBsAg), patients with hepatocellular carcinoma, patients with severe psychiatric disease and those with serious co-morbid conditions were excluded from the current study. Effective contraception and no breastfeeding were documented during the study period.

Enrollment and follow up data

A standardized enrollment questionnaire which was completed by patients' physicians at the initial evaluation and follow up visits were retrieved from patients' medical records. The initial questionnaire included medical number, full name and contact details for each patient in addition to the demographic data such as age, gender, body mass index (BMI), routine laboratory parameters in addition to the HCV-RNA results, grades of hepatic necro-inflammation and stages of fibrosis. Follow up visits included clinical and laboratory assessment to report possible adverse side effects and treatment response.

Patients were defined as having hematological abnormalities if they had the presence of either or a combination of the following hematological parameters at least once during follow up period; drop in hemoglobin (Hb) level <10gm/dl, absolute neutrophil count (ANC)<750 cells / mm3, and platelet(PLT)count<80 x 10^3/µL.

Data cleansing and setting high quality data

Data cleansing was applied for detecting, correcting or removing corrupt or inaccurate records from database in addition to the removing of typographical errors or validating and correcting values against a known list of entities. High quality data was characterized by accuracy, integrity, completeness, validity, consistency, uniformity and uniqueness.

Statistical analysis and tabulation of results

Data were processed and analyzed using SPSS (version 11.0.0; Statistical Package for the Social Sciences). Exploration of data using one sample kolmogrov.smirnov test revealed preserved

normality. Analysis of difference between two groups for these variables was done using unpaired t-test. Effect of therapy was done by the paired t-test. Therefore test for difference was Mann Whitney test and the effect of therapy was detected using Wilcoxon signed ranks test for this variable. All performed tests were two-tailed and considered to be statistically significant at the level of $p<0.05$.

RESULTS AND DISCUSSION

At week 48, end of treatment response (ETR) was evident in 2277 patients (61.6%), 1442 patients demonstrated failure of response and breakthrough including 492 patients (34.1%) who discontinued the treatment either due to side effect or non-compliance. At week 72 estimated SVR was 52.5%.

Initial hematologic parameters were not significantly related to the achievement of ETR among chronic HCV patients who had received PEG-IFN/RBV (Table1). However lower count of all parameters were associated with moderate or advanced hepatic fibrosis stages according to the METAVIR scoring ($p<0.001$).

During the 48 weeks of anti-viral therapy, anaemia(drop in Hb <10gm/dl) occurred in 31.8%, neutropenia(ANC)<750 cells / mm3) occurred in 22.3%, while thrombocytopenia(PLTcount<80 x 10^3/µL) occurred in 10.5%.

Univariate logistic regression analysis revealed that the drop in Hb, TLC and PLT count during the first month of combined pegylated-IFN/RBV therapy were significantly related to the achievement of SVR; p value < 0.01, 0.03,0.03 respectively while the drop of ANC did not show such significance ,p value>0.43 (Table 2).

As for the multivariate logistic regression analysis, the pattern in Hb level was the only hematological parameter that was significantly associated with SVR, odds ratio; 0.47, p value<0.001, Confidence interval (0.371-0.607).Other parameters such as patients; age, gender, viral load, AFP<10ng/ml and ALT >40IU/L were significantly related to the SVR as well.

Table (1): Comparison of baseline haematologic parameters between responder and non-responders in chronic HCV patients under PEG-IFN/RBV at 48 weeks.

Baseline Hematological parameter	Treatment Response		p value
	ETR	Non-ETR	
TLC(x10^3 cells / mm3)	6.48±1.82	6.43±1.79	0.359
ANC(cells / mm3)	3.41±1.21	3.35±1.23	0.188
Hb(g/dl)	14.13±1.49	14.08±1.55	0.334
PLT(x 10^3/µL)	213.8±61.16	212.8±63.89	0.642

(ANC): Absolute neutrophilic count. (ETR): end of treatment response. (Hb): hemoglobin. (PLT): platelet count. (TLC): total leucocytic count. p>0.05 (insignificant)

Table (2): The relationship between the drop of hematological parameters and the achievement of ETR and SVR to anti-viral therapy among chronic HCV patients.

Response	Drop of hematological parameters under combined pegylated-IFN/RBV			
	Hb<10 (g/dl)	**TLC<1500 (x10^{3}cells /mm3)**	**ANC<750 (cells / mm3)**	**PLT<80 (x10^3/µL)**
Week 48	n=335	n=1482	n=1710	n=1845
ETR n (%)	197(58.8)	940(63.4)	679(39.7)	1168(63.3)
P value	<0.01	<0.05	>0.14	<0.04
Week 72	n=283	n=1535	n=1693	n=1416
SVR n(%)	209(73.8)	542(35.3)	559(33)	457(32.7)
P value	<0.01	<0.03	>0.43	<0.03

(ANC): Absolute neutrophilic count. (ETR): end of treatment response. (Hb): hemoglobin. (PLT): platelet count. (TLC): total leucocytic count.

Egypt has the largest epidemic of chronic HCV in the world; the overall prevalence positive for antibody to hepatitis C virus in Egypt is 14.7% [2]**.** The most effective current standard of care treatment for chronic HCV infection is the combination of pegylated interferon (PEG-IFN) α-2a or α-2b and ribavirin (RBV) [11] **.**

Haematological abnormalities such as anemia, thrombocytopenia and leucopenia are common complications of HCV infection as well as during combination antiviral therapy[12] . Although dose reduction or discontinuation can easily treat these side effects, they can adversely affect the efficacy of combination antiviral therapy reducing the likelihood of a sustained viral response (SVR)[13]**.** Thus early identification and management of haematological side effects of antiviral therapy for HCV infection can be an important strategy for maximizing treatment outcomes.

In our series, as with all standard protocols, initial hematological parameters were within normal accepted ranges, thus their levels did not have any significant difference with respect to the achievement of therapeutic outcome. These ranges often enables the patients to continue the treatment without any dose modifications which my lead to drop in the response rate. During anti-viral therapy, anaemia, lecucopenia, neutropenia and thrombocytopenia were observed in 1181 patients (31.8%), 2237 (60.2%), 829 (22.3%), 390 (10.5%), were not significantly related to the type of IFN used. Previous studies have reported similar findings

In the present work, anaemia occurred in 31.8% regardless the type of IFN and this went hand in hand with **Chao et al. (2006)** [14]**, and Watson et al. (2010)** [15] who reported anaemia in 39% of their patients, but this was different from **Kaplan et al. (2009)** [16]**,** who reported anaemia in 9-13% only in their patients during anti-viral therapy. The occurrence of anemia in patients receiving HCV therapy often has multiple contributing factors, including ribavirin, interferon, the stage of HCV-chronic liver disease[17]**,** or due to the genetic predisposition. **Kamatani et al. in**

(2010)[18]**,** discovered that genetic variants that cause deficiency in the production of an enzyme called inosine triphosphate pyrophosphatase gene (ITPA) affects ribavirin-induced anemia.

During the first 4 weeks of anti-viral therapy, the drop of Hb<10g/dl,TLC<1500(x10^3cells /mm3), PLT<80(x10^3/µL) was significantly related to the achievement of ETR and SVR.

Similar records were obtained by **Hayat** and his colleagues **(2009)** [4]**,** who found significant anemia (Hb<10gm/dl) which was greater in women then men, suggesting absolute Hb concentration for ribavirin dose reduction may not take into account potentially important factor such as sex and the magnitude of the relative Hb decline. Similar observation was also noted by other authors, they also showed a drop of 2gm of mean Hb% at 6 months of therapy, but in there study all the patients were males[19]**.**

IFN can also contribute to the development of anemia by suppressing bone marrow production of erythrocytes[20]**.**

A common another important hematological side effect with HCV is thrombocytopenia[21]**.** Multiple mechanisms are implicated in the pathogenesis of thrombocytopenia induced by IFN. They include bone marrow suppression, immune-mediated destruction of platelets and rapid sequestration of PLT in the capillary beds of the liver and spleen[5]**.** IFN associated thrombocytopenia may be acute or chronic[22]**.**

Yamane et al. (2009)[23]**,** demonstrated that IFN did not inhibit colony formation of megakaryocytes from human CD34+ hematopoietic stem cells, neither did it inhibit endomitosis, but it did inhibit cytoplasmic maturation of megakaryocytes and the subsequent PLT production in vitro.

These studies stated that IFN suppresses the expression of transcription factors regulating late-stage megakaryopoiesis, such as GATA-1, p45NF-E2, Maf G. Reducing the number of the PLT in the peripheral blood but not megakaryocytes. In **2010 Stellacci et al**.[24] stated that IFN inhabit the maturation of demarcation membranes in megakaryocytes.

Autoimmune mechanism may be also involved[25]**,** or due to relative thrombopoietin signal transduction in megakaryocytes[26]**.** About 46% of patients receiving IFN and ribavirin experienced thrombocytopenia with platelets counts felling below 5o.ooo requiring dose reduction during treatment[27]**,** so the low baseline platelets count increase the incidence of thrombocytopenia associated with antiviral therapy[28]**.**

In the present work we selected the whole patients with normal platelets count from the start. Thrombocytopenia occurred in 10.5% of our patients and this was similar to the results that were reported by **Renou et al. (2005)**[27] who reported thrombocytopenia in about 10-15% of patients.

Neutopenia in patients with HCV receiving IFN and ribavirin therapy showed that 8%-24% of these patients develop neutropenia. They showed that absolute neutrophil and lymphocytes counts typically decreased by 30% to 50% of the baseline during therapy[28]**.** Though neutopenia is frequent during HCV treatment, it was not found to be commonly associated with infections[30]. We reported neutopenia in 22.3% of our patients during their treatment and this was similar to the previous studies in this issue.

CONCLUSION:

Initial hematological parameters were not predictors of treatment response; however; low Hb level, WBCs, ANC, and PLT count were associated with advanced stages of hepatic fibrosis. Anaemia, leucopenia and thrombocytopenia during anti-viral therapy were significantly associated with response rates regardless to the type of PEG-IFN.

ACKNOWLEDGEMENTS:

We honored to The Egyptian National Committee for Control of Viral Hepatitis and the Science and Technology Development Fund (STDF) for their help and support.

REFERENCES

1- Russo MW and Fried MW Side effects of therapy for chronic hepatitis C. Gastroenterology; 2003;124:1711–1719.

2- Solas C, Paré M, Quaranta S, Stanke-Labesque F. Evidence-based Therapeutic Drug Monitoring for Ribavirine. Therapies. 2011;66(3):221-230.

3- El Zanaty W, Hamid F, Esmail M. .Egypt demographic and health survey 2008.

4- Hayat AS, Khan AH, Shaikh N, Farzana M, Ghouri RA. Hematological Side Effects Following antiviral therapy (conventional alpha Interferon plus Ribavirin) in patients with Chronic Hepatitis C. World Applied Sciences Journal 2009; 6(8):1017-1020.

5- Wang Q, Miyakawa Y, Fox N.. Interferon-alpha directly represses megakaropoiesis by inhibiting thrompocytopenia induced signaling through induction of SOCS-1. Blood; 2005;96(5): 2029-2039.

6- Dieterich DT and Spivak JL. Haematologic disorders associated with hepatitis C virus infection and their management. Clin Infect Dis2008;37 (4): 533-541.

7- Everson GT, Trotter J, Forman L, Kugelmas M, Halprin A, Fey B, Ray C. Treatment of advanced hepatitis C with a low accelerating dosage regimen of antiviral therapy. Hepatology; 2005.42: 255-262.

8- De Franceschi L., Fattovich G., Turrini F. et al. Haemolytic anemia induced by ribavirin therapy in patients with chronic hepatitis C virus infection: role of membrane oxidative

damage. Hepatology, 2000;31: 997-1004.

9- EASL 47th Annual Meeting .Conference Reports for NATAP, April, 18th - 22nd 2012; Barcelona, Spain.

10- Bedossa P and Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR cooperative study group. Hepatology 1996; 24: 289-293.

11- Hadziyannis SJ, Morgan TR, Balan V. Peginteron-alpha and ribavirin combination therapy in chronic hepatitis C. Ann Intern Med. 2004;140 (7): 346-355.

12- Wong P, Hilzenrat N, Adfal NH, et al. Role of epoetin alpha in maintaining ribavirin dose. Gastroenterol Clin N Am 2009;22(4): S25-S35.

13- Mancino P, Falasca K, Ucciferri C, Pizzigallo E, Vecchiet J. Use of hematopoietic growth factor in the management of hematological side effects associated to antiviral treatment for HCV hepatitis. Mediterr J Hematol Infect Dis. 2010;2(1): 31-37.

14- Chao T, Meidinger RR, Kayali Z, et al. Incidence of ribavirin induced anemia in patients with chronic hepatitis C. Gastroenterology 2006;132, A790. (Abstract).

15- Watson J, Hassanein TI, Kayali Z. et al. prospects for hepatitis C virus therapeutics: Boceprvir and telaprevir as adjuvant therapy to the standard antiviral regimen. Curr Opin Investig Drugs 2010; 4 (6): 5705-5708.

16- Kaplan N, Fornasieri A, Giachino O, et al. Multicenter study on ribavirin-related haemolytic anaemia. Am J Blood Dis. 2009;46 (5): 69-82.

17- Alessandra M, John G, Mc Hutchison N, et al. Inosine triphosphatase genetic variants are protective against anemia during antiviral therapy for HCV2/3 but do not decrease dose reductions of RBV or increase SVR. Hepatology, 2011;36(3): 1273-1279.

18- Kamatani N, Nakamura Y, Miyakawa Y. influence of inosine triphosphatase gene (ITPA) polymorphism on decrease of heamoglobin during treatment with pegylated interferon, ribavirin and telaprevir. Hepatol 2010; 40 (5): 1450-1458.

19- Shahzad Saeed, Muhammad Irfan, Khattak, Tassawar Hussain. Effect of antiviral therapy on hematological parameters in patients with chronic hepatitis. Pak Armed Forces Med J 2006; 56(3):228-231.

20- Trivedi HS and Trivedi M. Subnormal rise of erythropoietin in patients receiving interferon and ribavirin combination therapy for hepatitis C. J Clin Gastroenterol. 2007;38 (3): 595-598.

21- Garcia-Suarez J, Pivetti S, Lepelletier Y, et al. HCV associated thrombocytopenia: clinical characteristics and platelet response after recombinant alpha 2b- interferon therapy. Br J Haematol; 2007;110(1): 98-103.

22- Lang AM and Norland AM. (2008): New view on pathogenesis of interferon induced

thrombocytopenia in patients with chronic hepatitis C. Proc Natl Acad Sci USA, 104 (12): 985-990.

23- Yamane A, Nakamura T, Suzuki H. et al Interferon-alpha-2b induced thrompocytopenia is caused by inhibition of platelet production but not proliferation and endomitosis in human megakaryocytes. Blood. 2009;112 (13): 542-550.

24- Stellacci E, Testa U, Petrucci E et al. Interferon inhibits maturation of demarcation membranes in megakaryocytes. Biochem 2010; J; 377 (8): 367-378.

25- Mihai Olariu, Cristina Olariu, Dan Olteanu. Thrombocytopenia in Chronic Hepatitis C. J Gastrointestin Liver Dis. 2010;19 (4), 381-385.

26- Peck-Radosavljevic M, Wichlas M, Pidlich J et al. Blunted thrombocytopenia response to interferon alpha induced thrombocytopenia during treatment for hepatitis C. Hepatology2007;28(5):142-149.

27- Renou C, Rosenthal E, Cohen P. et al.. Incidence of thrompocytopenia in patients with hepatitis C virus infection receiving interferon therapy. A prospective multicenter study of 321 patients. Hepatology, 2005;79 (13): 32-42.

28- Dourakis SP, Deutsch M, hadziyannis SJ. Thrombocytopenia and interferon therapy. J Hepatol 2006; 25(4): 972-975.

29- Antonini MG, Babudieri S, Maida I, et al. Incidence of neutropenia and infections during combination treatment of chronic hepatitis C with pegylated interferon alpha plus ribavirin. Infection, 2008;36 (4): 250-255.

30- Navas S, Martin J, Quirogaa JA. Recombinant human granulocyte colony stimulating factor reduces hepatitis C virus replication in mononuclear cells from chronic hepatitis C patients. Cytokine 2010;8 (7): 313-317.