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Folic Acid and Vitamin B Complex Improves Quality of Life in Hepatitis C Infected Patients Treated with Peginterferon and Ribavirin

Nermeen Ashoush[1*], Manal El-Hamamsy[2], Mona Schaalan[3], Osama Ahmed[4], Mohey Elmazar[1], Osama Badary[2]

1: Faculty of Pharmacy, The British University in Egypt.
2: Faculty of Pharmacy, Ain Shams University.
3: Faculty of Pharmacy, Misr International University.
4: Faculty of Medicine, Ain Shams University, Cairo, Egypt.

ABSTRACT

Pegylated-interferon α-2a and ribavirin (PIFN/RBV), the current standard treatment for hepatitis C virus (HCV) infection in Egypt, is frequently associated with hematological adverse effects, leading to high treatment discontinuation rates. The objective of the present study is to explore the effectiveness of intervening with folic acid (F) and/or vitamin B complex (B) compared with placebo (C) in HCV-treatment Egyptian patients for the management of treatment-induced deterioration of health related quality of life (HRQOL) as well as hematological parameter. In a randomized controlled trial, one hundred and sixty subjects were randomly assigned to receive PIFN/RBV in addition to BF, B, F, or C. Blood samples were collected at different time points during 48 weeks and at 12 and 24 weeks post treatment for complete blood count and for HCV RNA real time PCR. Short form SF 36V2 questionnaire were used to assess HRQOL at various time during and post treatment. Egyptian HCV patients treated with PIFN/RBV showed deterioration of HRQOL which were correlated with deterioration in the measured hematological parameter. Supplementation with vitamin B complex plus folic acid significantly ($P<0.001$) decreased the deterioration observed in physical and mental health as well as complete blood count. Supplementation with either vitamin B complex or folic acid were also effective but with lower potency than their combination. BF supplementation can reduce adverse effects of PIFN/RBV therapy in chronic hepatitis C patients, which may improve patients' HRQOL and their adherence to combination antiviral therapy.

Keywords: Hepatitis C virus, Quality of life, Folic acid, Vitamin B complex, Peginterferon, Ribavirin.

*Corresponding Author Email: nermeen_ashoush@hotmail.com
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INTRODUCTION

Chronic hepatitis C often gradually progresses to cirrhosis, hepatocellular carcinoma and advanced hepatic fibrosis. Ribavirin has been used in combination with pegylated interferon (PIFN/RBV) as the most effective antiviral therapy[1].Although this PIFN/RBV therapy seems effective and safe for hepatitis C, several side effects such as anemia, neutropenia, leukopenia, thrombocytopenia, depression, and flu-like syndrome from this anti-hepatitis C virus therapy have been reported[2]. Hepatitis C virus (HCV) patients commonly experience fatigue, anxiety, and depression. These symptoms negatively affect patients' health related quality of life (HRQL) and well-being. In addition to well-known side effects of interferon, one important determinant of HRQL during anti-viral therapy for HCV is development of ribavirin-induced anemia. There is some evidence that HCV patients who experience poorer HRQL are more likely to discontinue treatment prematurely with its negative impact on virologic response[3]. Treatment of anemia improves HRQL, potentially impacting adherence to antiviral regimen and improving virologic response[4]. Hepatitis C virus infection and PIFN/RBV led to the decline of B vitamins including vitamin B1, B6, vitamin B12 and folic acid. it has been documented that B vitamins could exhibit antioxidant activity *via* scavenging oxygen radicals and organic radicals. Thus, the decrease in B vitamins diminished their antioxidant defense[5]. On the other hand, the depletion of B vitamins also favored the occurrence of anemia in HCV patients with PIFN/RBV therapy. It was speculated that the antioxidant effect of B vitamins could protect the erythrocyte membrane from the RBV-induced hemolysis and would promote erythropoies is in response to the RBV-induced anemia[6]. In the present study, it was aimed to investigate the beneficial effect of folic acid and vitamin B complex supplementation on the PIFN/RBV-induced anemia and HRQOL by conducting a randomized controlled trial.

MATERIALS AND METHOD

Patients

The study population consisted of treatment-naive patients with chronic hepatitis C who were enrolled into a randomized, prospective, single blind, placebo-controlled trial conducted at Hepatology and Gastroenterology Units at Ain Shams University Hospitals in the period from September 2012 to March 2014. Randomization was performed by using block of four that was randomization using a computer-generated table. Patients were blinded to the true identity of medications. The inclusion criteria included elevated serum alanine amino transferase at least two times above the upper limit of normal (ULN: 40 U/l) during the preceding 6 months, detectable anti-HCV antibody status assessed by a second-generation enzyme-linked immunosorbent assay (Roche Diagnostics, NJ, USA), detectable HCV RNA

by a polymerase chain reaction (Cobas Amplicor HCV monitor, Roche Diagnostics), an infection with HCV-4 [Versant HCV genotype assay (LiPA) 2.0; Innogenetics, Eschborn, Germany]. Patients were excluded if they had received previously an IFN-α-based regimen or showed evidence of other liver diseases, including hepatitis A, hepatitis B, autoimmune hepatitis, alcoholic liver disease, drug-induced hepatitis and decompensate liver disease, coinfection with schistosomiasis or HIV, leucocyte count <3000/mm^3, neutropenia (<1500 cells/mm^3), hemoglobin level <12 g/dl for women and <13 g/dl for men, thrombocytopenia (<90 000 cells/mm^3), creatinine concentration 1.5 times above ULN, organ transplantation, malignant conditions, severe cardiac or pulmonary disease, unstable thyroid dysfunction, severe depression or psychiatric disorder, active substance abuse, current pregnancy or breast feeding, known sensitivity to the drugs tested or therapy with immunomodulatory agents within the last 6 months. Subjects were also excluded if they were considered by the investigators to be potentially unreliable, non-compliant to therapy or unwilling to apply birth control during therapy.

Study Design

The current study was a prospective, randomized, single blind, placebo controlled clinical trial. One hundred and sixty eligible patients were randomized in blocks of four (each group of 40 patients) to receive pegylated-interferon α-2a (Pegasys®, Hoffmann-LaRoche) at a dose of 180 µg/week plus ribavirin (Ribavirin®, Schering-Plough Corporation). The ribavirin dose ranged between 1000 and 1200 mg/day according to body weight (<75 kg, 1000 mg/day and >75 kg, 1200 mg/day respectively). All patients received treatment for 48 weeks. Patients in Group B received a tablet of vitamin B complex (Neurobion®, Glaxosmithkline, Egypt) (each tablet contains 100 mg of Vitamin B1, 200 mg of Vitamin B6 and 1 mg of Vitamin B12) three times per day during treatment with PIFN/RBV. On the other hand, Group F received one capsule of 5 mg of folic acid (Folic acid®, NileCo, Egypt) twice daily to take with PIFN/RBV. Group BF received one capsule of folic acid twice daily and a tablet of vitamin B complex three times per day during treatment with PIFN/RBV. Group C received matching placebo capsule and/or tablet to take with PIFN/RBV. The vitamin supplemented patients received folic acid and vitamin B complex with the first dose of PIFN/RBV and throughout the 48 weeks of study. Participating patients were given written informed consent and received a copy of their rights at enrollment and before any study-related procedure. The study was approved by the Institutional Ethics Committee (FMASU 1669). This trial was registered in clinical trial.gov ID: NCT02150291. The study was conducted according to the guidelines of declaration of Helsinki. Patients visited the participating center once a week for 4 weeks, once every 4 weeks thereafter and 12 and 24 weeks after the end of therapy. During

these visits, physical examination was performed, reviewed the patients' blood picture and liver functions and evaluated adherence through an open-ended questionnaire. HCV RNA levels were evaluated during pretreatment screening, at baseline, at treatment weeks 4, 12, 24 and 48 and at week 24 post-treatment.

Blood sampling and laboratory tests

At each visit, approximately 5mL of blood was taken from patients for hematological, biochemical, and virological assessments. The patient's white blood cell and platelet counts, aspartate aminotransferase and alanine aminotransferase, albumin, hemoglobin, bilirubin, blood urea nitrogen, and creatinine level as well as prothrombin time were measured using the standard laboratory techniques used by the hospitals.

Measurements and outcomes

The primary efficacy endpoint was to maintain absolute neutrophil count levels >750 cells/mm^3, hemoglobin levels >10.5 g/dL, platelet count above 45,000 platelet/mm^3 to prevent adjustment of the PIFN/RBV dose or its temporary suspension[7].Criteria for dose down of ribavirin or stopping of folic acid and vitamin B complex were as follows: dose-down of ribavirin from 1200 to 800 mg/day when hemoglobin level less than 9 g/dl or absolute neutrophil count <0.8 x10^3/µl. Furthermore, folic acid and vitamin B complex administration will be stopped and shift to Eprex® (Janssen-cilag) (4000 IU SC every other day/week) and Neupogen® (Hoffmann-LaRoche) (300 IU SC once/week). The dose of ribavirin was restored to the original level if the adverse events were resolved or improved following dose reduction or growth factor administration. Patients will then be excluded from the study. During therapy, thrombocytopenia was assessed at levels of 50,000 and 25,000/µl, since these levels are the usual thresholds for dose reductions or discontinuation of PIFN/RBV therapy, respectively[8]. The secondary efficacy endpoint was sustained virological response (SVR) defined as undetectable serum HCV RNA (<615 IU/ml) by Roche Cobas®TaqMan® (Roche Diagnostics) 24 weeks after the completion of treatment. Other 'on treatment' viral kinetics included rapid virological response, defined as undetectable HCV RNA after 4 weeks of therapy, complete early virological response (EVR) defined as undetectable HCV RNA after 12 weeks of therapy and partial EVR defined as 2log10 decline in serum HCV RNA after 12 weeks of therapy and end-of-treatment response (ETR), defined as undetectable serum HCV RNA (<615 IU/ml) using Cobas®TaqMan® at the end of the scheduled treatment. Patients were considered non-responders and their therapy was discontinued if they had detectable HCV RNA or a minimal change in HCV RNA titers (a decrease of <2 log10 IU from the baseline level) after 24 weeks of treatment.

Assessment of safety

At each follow up appointment, the patients in each group will be questioned about any possible adverse effect related to treatment. Safety was assessed through monitoring of patient reported adverse events and assessing clinical and laboratory test results.

Health-related quality of life assessment

Patients were invited to complete the Arabic version of the Short form-36 Health Survey version 2 (SF-36v2) questionnaires. The SF-36 questionnaire includes 8 scores measured on a scale 0–100 with a 100 indicating the best possible health status: physical functioning, role physical, bodily pain, general health, vitality, social functioning, role emotional, and mental health. The two summary scores, namely, Physical Component Summary score (PCS) and Mental Component Summary score (MCS) summarize the physical and mental health components of SF-36. To eliminate a possible effect on perceived quality of life, patients were blinded to patient's HCV RNA level measured at the time of each visit.

Statistical analysis

Analysis of the data was performed using SPSS (Statistical package for social science) computer program version 21. The data were expressed as mean ± standard deviation. When comparison was between more than two means repeated measures (Split-Plot), using difference from baseline analysis of variance (ANOVA) followed by post-hoc Bonferronitest was carried out. P-value ≤0.05 was considered significant. The results of the summary scores of SF-36 were presented as means. Patients were also divided to three categories those with a summary scores of SF-36 <30, 30-<50, and ≥50. The frequencies of the three categories/group were tested using chi-square test or fisher's exact test where appropriate.

RESULTS AND DISCUSSION

Combination antiviral therapy with pegylated-interferon α-2a or α-2b with ribavirin (RBV) has become the mainstay of current HCV therapy in Egypt. The induced hematologic side effects, however, have become a serious medical issue requiring proper management. Anemia is the most frequently reported hematologic abnormality that is classified as ribavirin-induced hemolytic anemia and IFN–induced bone marrow suppression. Anemia is reported to be associated with decreased life quality and is considered a limiting factor during therapy, raising consideration for dose reduction or treatment discontinuation[9]. One hundred and sixty patients (138 males and 22 females) with proven chronic HCV-4 were considered eligible and consented to enroll in the study. There were no significant differences between treatment groups for any baseline demographic or clinical characteristics (Table 1). The mean ± SD age of the entire cohort was 29.6 ± 6.3 years for females and 30.9 ± 7.2 for males, with the latter constituting 86% of the cohort and their mean body mass index was 51.63 Kg/m^2. There was

no evidence of cirrhosis detected by ultrasonography in all patients included in the study. Seventeen patients (12 males and 5 females) were excluded during the treatment because of retinopathy (n=1), insufficient therapeutic response at week 12 (n=7), those who had received epoetin during antiviral therapy (n=9).Of the 160 patients enrolled in the study, data of only 143 patients, who had completed 48 weeks of treatment, were analyzed. Seven patients were withdrawn prematurely, mostly at week 12, due to insufficient therapeutic response. Sustained virological response (at 24 weeks post-treatment) was shown to be 86.1% (31/36), 73% (27/37), 80% (28/35) and 65.7% (25/35) of patients in group BF, B, F and C; respectively (Table 2). There was no statistically significant difference between groups as regards SVR (P= 0.4168), RVR (P=1), cEVR (P=0.5375) and pEVR (P=0.5375). On the other hand, a statistically significant difference was found between groups as regards ETR (P= 0.0304). Patients were asked about specific side effects via semi-structured questions. As anticipated, the most commonly reported physical side effects of treatment were flu-like symptoms with chills, fever, arthralgia, myalgia, rigors, headache, fatigue and weakness. Altogether, flu-like symptoms were experienced by almost all patients under therapy which was more severe after the first injections of PIFN/RBV; tended to abate after 4–6 weeks from start. A prominent finding in the current study was the potential fatigue that can impair multiple domains of life that mostly evident in all groups starting from the first week of therapy, especially in group C receiving PIFN/RBV, due to concomitant severe anemia.. Adverse effects increased during the first four weeks of therapy and remained at this higher level for the duration of treatment. Once therapy was terminated, adverse effects decreased. Interestingly, in patients in group BF, and due to hematological improvement, the manifestation of fatigue was less prominent than the control group. In patients with chronic hepatitis C, who are treated with PEGIFN/RBV therapy, the development of fatigue is often initially considered to be an IFN-related adverse event. Fatigue or an element thereof may also be attributed to ribavirin-related anemia. However, as IFN can contribute to ongoing fatigue, the symptom and associated impairments may not resolve solely with interventions given to restore normal Hb concentrations[10]. Sub-conjunctival hemorrhage, retinal hemorrhage, and cotton wool spots have been reported that required treatment discontinuation in one patient. Egyptian HCV patients treated with PIFN/RBV showed deterioration of HRQOL. These deteriorations were observed as early as the first week of treatment. It recovered slowly thereafter, but it did not reach pretreatment values until week 24. These deteriorations were correlated with deterioration in the measured hematological parameter such as total leukocyte count, absolute neutrophil count, platelet and hemoglobin level, but their values were progressively decreased during the whole 48 weeks of treatment.

At baseline, mean scores for physical component summary (PCS) and mental component summary (MCS) for the four treatment groups fell somewhat below normative values (mean scores of 50 for each summary scale) for the population. The baseline SF-36 scores did not differ significantly by treatment groups except group B compared to control group, a statistically significant difference was found as regards PCS (P=0.0498) and MCS (P=0.0111). During the first 4 weeks of therapy, there was a decrease in Physical Component summary. Most of the patients in the four treatment groups have PCS score <30. There was a statistically significant difference between the patients' PCS score in group BF (P=0.005) compared to control group C. No statistically significant difference was found between PCS score of patients in group F (P=1), group B (P=1) compared to the control group. Moreover, there was a statistically significant difference between patients in group F (P=0.0278), group B (P=0.002) compared to patients receiving group BF in addition to antiviral therapy (Figure 1). At week 24, PCS score began to increase above 30 in the four treatment groups. A statistically significant difference was found between patients' PCS scores in group BF (P=0.0113) compared to the control group C. However, no statistically significant difference was found between patients' PCS scores in group F (P=1) and group B (P=1) compared to patients receiving placebo group C (Figure 1). At week 48, there were more patients scoring 50 or above in group BF (P<0.0001) compared to group C. On the other hand, no statistically significant difference was found between patients' PCS score in group F (P=0.2391) and group B (P=1) compared to group C. However, a statistically significant difference (P<0.0001) was found in group F and group B compared to the group BF (Figure 1). Patient's physical component summary score rapidly returned to normal population average score at 12 and 24 weeks after treatment discontinuation. Mental component summary began to decline 4-8 weeks after starting therapy in all treatment group. At week 4, most of patients' MCS score was less than 30 except patients in group BF. There was a statistically significant difference between patients' MCS score in group BF (P<0.0001), group B (P=0.0007), and group F (P<0.0001) compared to control group C. Moreover, a statistically significant lower score was found between patients in group F (P=0.0002) and group B (P=0.0001) when compared to patients in group BF (Figure 1). At week 24, patients' MCS score started to increase and the majority of patients in group BF was above 50 compared to other treatment groups. There was a statistically significant higher MCS score in patients in group BF (P<0.0001) compared to control. A statistically significant difference was found between patients' MCS score in group F (P<0.0001) and group B (P<0.0001) compared to control group C (Figure 1). At week 48, there were statistically significant more patients in group BF whose MCS score was above 50 than other three groups (P<0.0356). There was a statistically

significant higher MCS score of patients in group BF (P<0.0001) and group F (P<0.0001) compared to group C. On the other hand, no statistically significant difference was found between patients in group B compared to group C (P=0.1981) (Figure 1). The present study confirms that having depression, fatigue, or insomnia during treatment are the major independent predictors of lower HRQL score at all-time points, from baseline to the end of treatment follow-up[11]. The results in the present study showed that patients treated with vitamin B complex plus folic acid in addition to anti-HCV regimen had a milder decline in their physical and mental health component during treatment. Evidence suggests that folate deficiency is causatively linked to depressive symptoms because folate plays an important role in the one-carbon metabolic pathway involved in methylation processes and the synthesis of neurotransmitters in the central nervous system[12]. Elevated homocysteine levels, a marker of folate deficiency as well as vitamin B12 deficiency, can also cause oxidative stress, resulting in cerebral vascular, neurological damage and neurotransmitter deficiency[13]. Low levels of serum or red blood cell folate, serum vitamin B12, dietary intake of folate and vitamin B12, and high levels of serum homocysteine are associated with an increased risk for depression. In addition, depressed people with low serum folate levels are significantly less likely to respond to some antidepressant medications (such as fluoxetine) and more likely to relapse during treatment[14]. Folate deficiency has been consistently associated with evidence of depression and cognitive decline, whereas low vitamin B12 concentrations have been mainly associated with cognitive impairment. Therefore, combination of folic acid and vitamin B complex are essential for improving patients mental component summary score compared to other treatment groups[15]. Although there was a mild decline in HRQL (physical and mental component summary) with patients receiving vitamin B complex plus folic acid in addition to antiviral therapy, the extent of this decline was substantially higher for those receiving folic acid or vitamin B complex alone or placebo. These data support the better tolerability of folic acid plus vitamin B when compared to each one alone, which is expected to result in better adherence to treatment and potentially better effectiveness of this regimen in the clinical practice setting. During the treatment period, patients in group BF demonstrated less declines in total leukocyte count and absolute neutrophil count relative to those receiving placebo, however only a modest decrement was noted in group F and group B. Nevertheless, after 12 weeks of follow up after stopping therapy, the levels recovered to baseline (Figure 2). By using repeated measures (Split-Plot) ANOVA, a statistically significant difference (P=0.000) was found between patients in group BF, group B, group F and group C as regards to total leukocyte count and absolute neutrophil count from baseline during 48 weeks of treatment. The pattern of decline in total leukocyte count and absolute

neutrophil count was also significantly different with respect to time as shown in Figure 2. Following the initiation of PIFN/RBV, absolute neutrophil count and total leukocyte count started to decrease rapidly within 2-4 weeks after initiation of treatment, with most of the decline occurring by treatment week 48. Twelve weeks after treatment discontinuation the absolute neutrophil and total leukocyte counts returned to pretreatment levels. As regards differences between groups in total leukocyte count and absolute neutrophil count during treatment period of 48 weeks, there was a statistically significant difference between patients in group BF (P=0.000, P=0.000) and patients in group F (P=0.022, P=0.038), respectively compared to those receiving placebo. On the other hand, no statistically significant difference was found between patients in group B (P=0.312, P=0.156), respectively compared to those receiving placebo group C. Moreover, there was no statistically significant difference between patients in group F (P=0.278, P=0.067), respectively compared to patients in group BF. On the other hand, there was a statistically significant difference between patients in group B (P=0.015, P=0.011) and patients in group C (P=0.000, P=0.000), respectively compared to patients in group BF (Figure 2). In the current study, a significantly greater decrease in total leukocyte count and absolute neutrophil count in patients treated with placebo in addition to PIFN/RBV was observed compared to patients treated with vitamin B complex and folic acid. This is in accordance with the study of McHutchisonet al.[16], in 3070 patients with HCV. The proportion of patients with neutropenia who met the criterion for PIFN dose reduction were: 21.1% receiving PIFNα-2a, 19.4% receiving standard dose PIFNα-2b, and 12.5% receiving low dose PIFNα-2b. Between 2.1 and 5.9% of patients met the discontinuation criterion based on neutropenia. In the present study, vitamin B complex plus folic acid improved neutrophil count by inhibiting leukocyte apoptosis possibly through its free radical scavenging and antioxidant properties[17]. Scagliottiet al.[18]reported that pemetrexed (a chemotherapeutic drug) was well tolerated, particularly in patients who received low-dose folic acid and vitamin B12. Supplemented patients had a marked reduction in hematologic toxicity, specifically grade 3/4 neutropenia and leucopenia as well as a suggested improvement in signs and symptoms of toxicity. The current study showed hemolytic anemia with various degrees within Egyptian patients receiving PIFN/RBV during the 48 weeks of treatment. Patients treated with PIFN/RBV (group C) had a pronounced decrease in hemoglobin level during the first 2–4 weeks of therapy. This effect was reversed 12 weeks after discontinuation of treatment. Hemoglobin concentrations were reported to decrease by an average of 2–3 g/dl during treatment with PIFN/RBV[19]. The effect was proved to be due to ribavirin (RBV) which produces a dose-dependent hemolytic anemia that is reversible within 4–8 weeks of drug discontinuation. The pathogenesis of RBV-induced

anemia is reported to be the destruction of erythrocytes due to the retention of RBV inside the erythrocytes because there is no RBV dephosphorylase in erythrocytes, possibly leading to the oxidative damage to the erythrocytic membrane[20]. Furthermore, Ribavirin-independent hematologic effects were also witnessed in patients receiving this drug in combination with IFN. This could be rationalized by the suppressing effect of IFN to bone marrow hematopoiesis which is associated with anemia, neutropenia, and thrombocytopenia[21]. Unfortunately, treatment with PIFN/RBV is associated with many side effects. One of these side effects is thrombocytopenia which accounts for a considerable number of dose reductions of peginterferonalfa to prevent (major) bleedings. These dose reductions compromised treatment efficacy[22]. The etiology of HCV-related thrombocytopenia is obscure. A thrombokinetic study revealed a significant, nearly linear, delayed splenic accumulation; normal or low-normal mean platelet life span; low-normal recovery; depressed platelet production; and a normal or slightly elevated mega karyocyte count in the bone marrow[23]. Several authors have reported the presence of HCV-RNA in peripheral platelets, suggesting direct infection of the platelets as the cause of increased destruction[24]. Patients in groups BF, B and F showed a significant higher level in hemoglobin (all at P=0.000) and platelet count (P= 0.000, P=0.029, P=0.019, respectively) compared to group C. On the other hand, hemoglobin and platelet count was statistically lower in group B (P=0.000, P=0.000) and F (P=0.000, P=0.000), respectively compared to group BF. Nevertheless, at 12 weeks after stopping antiviral therapy, the decrease in hematological parameter from baseline was successfully recovered in the four treatment groups (Figure 2). A total of 9 (5.62%) patients (Group BF=1, Group B=2, Group F=2, Group C=4) did not respond to folic acid or vitamin B complex and had developed severe anemia (<9 g/dl) and neutropenia (<0.8 $x10^3$/µl). Patients stopped therapy with folic acid or vitamin B complex at different week intervals during treatment, and received erythropoeitin (Eprex®) 4000 IU SC every other day/week and granulocytes growth factor (Neupogen®) 300 IU SC once/week and the dose of ribavirin was reduced to 800 mg daily which was associated with a mean hemoglobin increase of 11 g/L, at 4–8 weeks after treatment. In the present study, a significantly greater decrease in hemoglobin levels in patients treated with placebo in addition to PIFN/RBV was observed compared to patients treated with folic acid and vitamin B complex in addition to the PIFN/RBV. Decreased hemoglobin levels represent a common side effect of the PEGIFN/RBV used to treat HCV infection. This is in accordance with the study of Sulkowskiet al.[25] where 54% of patients on this regimen experienced hemoglobin decreases by 3g/dL or more from pre-treatment levels. Moreover, in a large clinical trial of Fried[26], the treatment-induced anemia (defined as Hb<12 g/dL) resulted in a RBV dose reduction in 22%

of patients and, in a community-based setting, anemia resulted in the discontinuation of therapy in 36% of patients. HCV infection leads to declined levels of vitamin B6, vitamin B12 and folate in HCV patients, as reported by Talwaret al.[27]. Furthermore, PIFN/RBV therapy not only leads to the decrease in vitamins B1 and B2 in plasma and/or RBCs but also exacerbate the depletion of vitamin B6**.** Vitamin B12 is an essential cofactor in at least two key transmethylation reactions, one of which closely interrelates with folate in DNA synthesis and hematopoiesis. The conversion of homocysteine to the amino acid methionine requires a B12-dependent enzyme as well as a methyl group donated by the folate compound 5-methyltetrahydrofolate (5-methylTHFA). With deficiency of vitamin B12, the enzyme function is disrupted, methionine formation is impaired, and both 5-methylTHFA and homocysteine accumulate. Through either, the trapping of folate in the form of 5-methylTHFA or the failure of methionine synthesis, the levels of the folate compound 5,10-methyleneTHFA are reduced, ultimately leading to impaired synthesis of thymidine. An inadequate supply of thymidine, in turn, impairs DNA synthesis, potentially leading to megaloblastosis and anemia[28]. Vitamin B1 and B6 have been related to the development or treatment of anemia during deficiency and supplementation, respectively. Vitamin B6 deficiency can disturb haeme synthesis and lead to normocytic, microcytic or sideroblastic anemia. Treatment of sideroblastic anemia with vitamin B6 has resulted in restored activity of erythroblastic d-aminolevulinic acid synthetase, the rate-limiting enzyme in haeme synthesis, followed by correction of the hematological abnormalities[29]. Although folic acid plus vitamin B complex possess a hematopoietic action, it can be speculated that the anemia-reducing effect of folic acid plus vitamin B complex was derived from its antioxidant and stabilizing potential of the erythrocyte membrane, thereby ameliorating, the hemolytic anemia particularly induced by RBV[30]. In the current study, patients treated with vitamin B complex plus folic acid in addition to antiviral therapy demonstrated less decline (P=0.000) in platelet count relative to those receiving placebo. Vitamin B complex and folic acid may modulate platelet activation through antioxidant mechanisms and the inhibition of the arachidonic acid cascade[31]. As regards levels of alanine aminotransferase and aspartate aminotransferase (data not shown), there was no statistically significant difference between patients in group BF (P=0.086, P=1), group B (P=1, P=1) and group F (P=0.207, P=1), respectively compared to those receiving placebo group C. The levels of plasma total bilirubin, thyroid stimulating hormone and creatinine were similar among HCV patients in the four treatment groups, and therapy did not affect the levels of these biomarkers. Noteworthy, Folic acid plus vitamin B complex did not affect the anti-viral effect of PIFN/RBV therapy in the present study.

However, if the hematological adverse effects could be improved and discontinuation of therapy could be avoided, it would lead to increased SVR in treated HCV patients[32].

Table 1: Baseline demographics and clinical characteristics of the 160 recruited patients

Variable	
Age in years, mean ± SD (range)	
• All	31 ± 7.07 (19-50)
• Male	30.9 ± 7.2 (19-50)
• Female	29.6 ± 6.3 (19-40)
Gender distribution (Male) n(%)	138 (86%)
Body mass index, in Kg/m^2, mean ± SD (range)	51.63 ± 5.25 (39.44-66.04)
HCV RNAnumber; mean ± SD (range)	1,393.179 ± 960,165 (39431-4165867)
Baseline Total Leukocyte Count (x10^9/L), mean ± SD (range)	5.85 ± 1 (4-10.9)
Baseline Absolute Neutrophil Count (x10^3/µL), mean ± SD (range)	2.58 ± 1 (1.6-6.51)
Baseline Hemoglobin Level (g/dl), mean ± SD (range)	
• All	14.7 ± 1 (13-16)
• Men	14.8 ± 0.7 (13.5-17)
• Women	14.7 ± 0.6 (13-15.4)
Baseline platelet count (x10^3/L), mean ± SD (range)	211 ± 17 (140-270)
Serum ALT Level (U/L), mean ± SD (range)	75 ± 24 (24-191)
Serum AST Level(U/L), mean ± SD (range)	62 ± 21 (24-130)
Creatinine Level (mg/dl), mean ± SD (range)	1.08 ± 0 (0.7-1.3)
Total Bilirubin Level (mg/dl), mean ± SD (range)	0.61 ± 0 (0.01-1.09)
Thyroid Stimulating Hormone Level (µIU/mL), mean ± SD (range)	2.26 ± 1 (0.2-4.8)
Mode of infection, n (%)	
• Past parenteral antischistosomal therapy	80 (50%)
• Injection drug use	20 (12.5%)
• Post-transfusion	12 (7.5%)
• Occupational	18 (11.25%)
• Unknown	30 (18.75%)

Table 2: Secondary outcomes in the investigated four treatment groups

Outcome	Group C (n=35)	Group B (n=37)	Group F (n=35)	Group BF (n=36)	P value
Sustained virological response, n (%)	25 (65.7%)	27 (73%)	28 (80%)	31 (86.1%)	0.4168
Rapid virological response, n (%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1.000
Complete early virological response, n (%)	7 (20%)	8 (21.6%)	6 (17.1%)	7 (19.4%)	0.9719
Partial early virological response, n (%)	25 (71.4%)	28 (75.7%)	23 (65.7%)	29 (82.9%)	0.5375
End of treatment response, n (%)	25 (71.4%)*	30 (81.1%)	33 (94.3%)	33 (91.7%)	0.0304
Non responders, n (%)	0 (0%)	0 (0%)	0 (%)	0 (0%)	1.000
Relapse, n (%)	2 (5.7%)	3 (8.6%)	3 (8.6%)	2 (5.7%)	0.9388

Results are expressed as number of patients in each group n (%)

* $P<0.05$ compared with group C

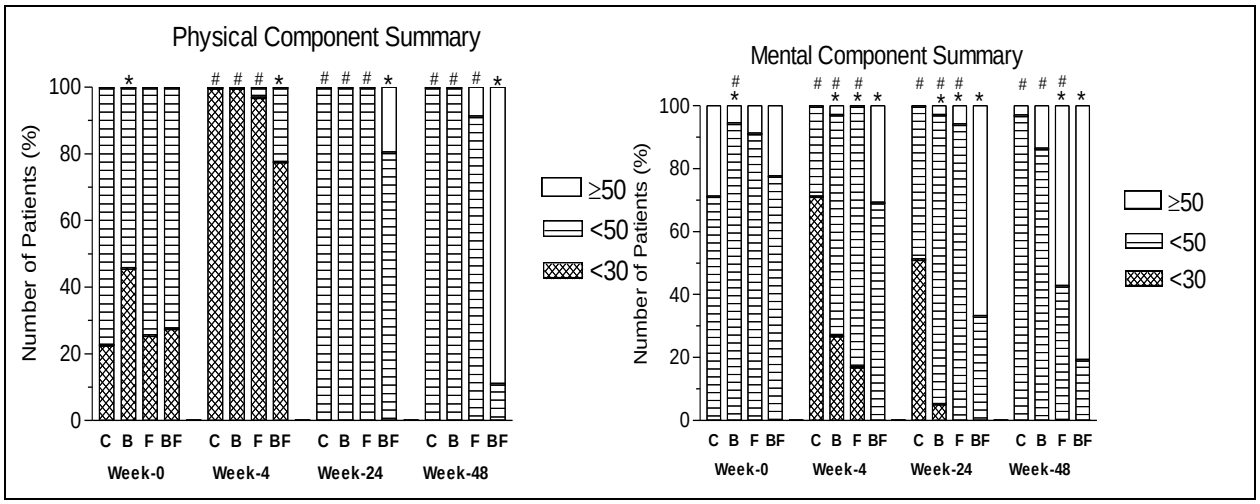

Figure 1: Distribution of the four treatment groups according to their physical component summary (PCS) and mental component summary (MCS) for 48 weeks.

HCV infected patients received PIFN/RBV before (week 0) and during administration of vitamin B complex (B), folic acid (F) and their combination (BF) for 48 weeks compared with control (C) (* P<0.05 compared with group C, $^{\#}$ P<0.05 compared with group BF). The number of patients in groups F and C= 35 while group BF=36 and group B=37. Patients with mean score <30 were considered worse, from 30-<50 were considered average and ≥50 were considered with normal physical or mental health.

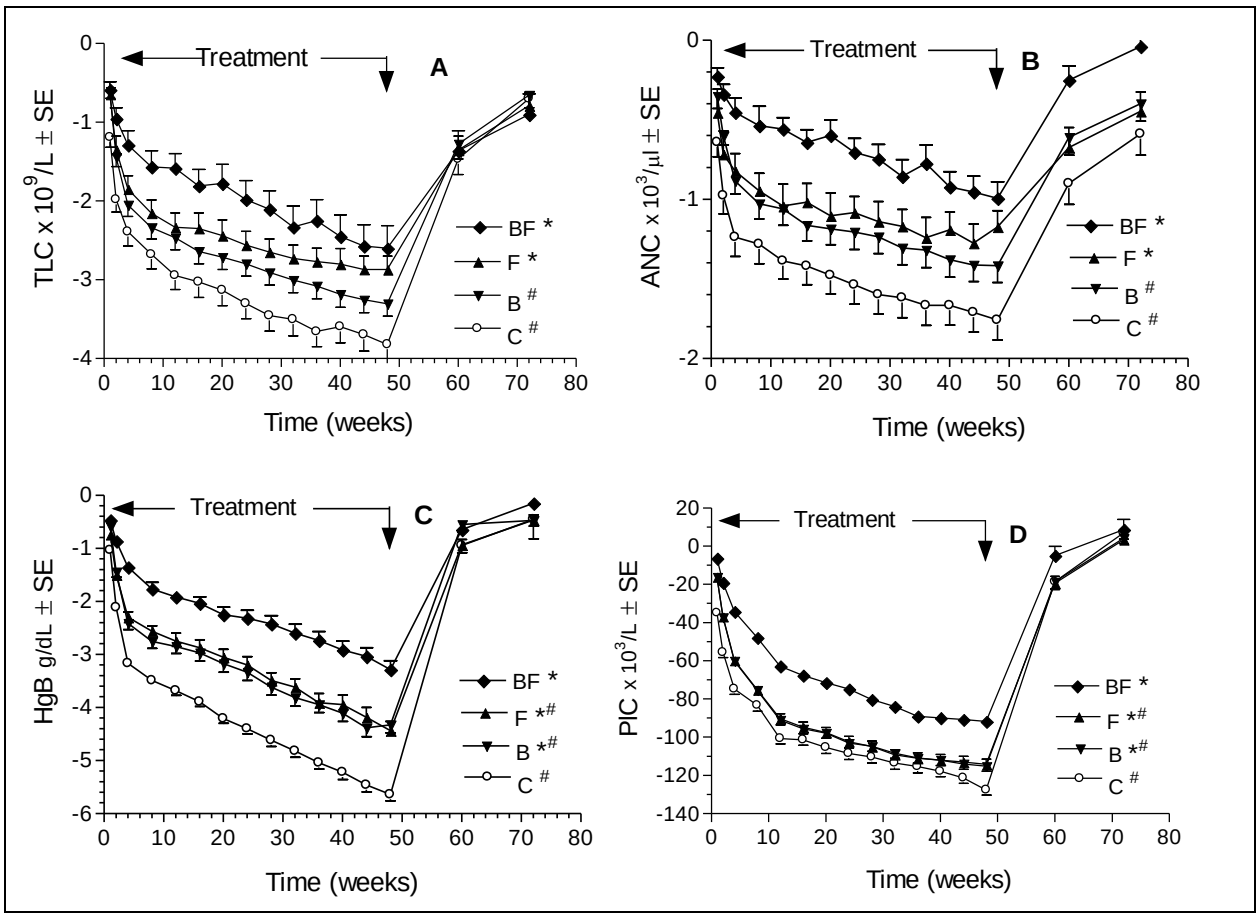

Figure 2:Change in hematological markers throughout the study period.

Change in total leukocyte count [A], absolute neutrophil count [B], hemoglobin level [C] and platelet count [D] from baseline in HCV infected patients before, during and after therapy with PIFN/RBV in combination with placebo (Group C), vitamin B complex alone (Group B), folic acid alone (Group F) and vitamin B complex plus folic acid (Group BF). The number of patients in groups F and C= 35 while group BF=36 and group B=37. (* significantly different ($P<0.05$) compared to control group during the whole treatment period, $^{\#}$ significantly different ($P<0.05$) compared to BF group during the whole treatment period.)

CONCLUSION

In conclusion, it was demonstrated that vitamin B complex plus folic acid supplementation can reduce some adverse side effects of PIFN/RBV therapy, but has no effect on SVR in chronic hepatitis C patients. Abatement of side effects may improve patients' health-related quality of life and their adherence to combination antiviral therapy.

Conflict of Interest

The authors declare no conflicts of interest. Neither the authors nor their organization have received any type of funding, incentives or in-kind payments and donations from pharmaceutical companies.

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