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Original Article

THE EFFECTS OF DIABETES MELLITUS ON THE RESPONSE TO PEGINTERFERON-ALPHA IN COMBINATION WITH RIBAVIRIN THERAPY IN EGYPTIAN CHRONIC HEPATITIS C PATIENTS

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ABSTRACT

Objective: The present study aimed to determine whether type 2 diabetes mellitus influences the response to antiviral therapy with peg-interferon alpha plus ribavirin in Egyptian patients with chronic hepatitis C.

Methods: All patients were treated with peginterferon alpha 2 b (1.5 µg/Kg/body weight) subcutaneously plus oral ribavirin application in a dose ranging from 800-1200 mg/day and followed after 12 w of therapy.

Results: The present study indicated that, non-significant changes were observed in liver function, kidney function, thyroid function tests, tumor marker, immunological analysis, hematological parameters, viral load and degree of cirrhosis between both groups' baselines, while the only significant difference was regarded in glucose level. However, diabetic group showed a significant decrease in response to antiviral therapy as compared to non-diabetic hepatitis C virus (HCV) patients. Furthermore, significant decrease in serum liver enzymes activity and total bilirubin level as compared to baseline levels in both groups, while there were a significant increase in alanine transaminase (ALT) activity and total bilirubin level in diabetic group as compared to non-diabetic HCV group after treatment. Also, significant decreases in hemoglobin concentration, white blood cells and platelet counts, in both groups after treatment as compared to there before treatment while diabetic group showed significant decreases in hemoglobin concentration and white blood cells count when compared with non-diabetic HCV group after treatment.

Conclusion: Type 2 diabetes mellitus influence the response to antiviral therapy with peginterferon plus ribavirin in Egyptian patients with chronic hepatitis C.

Keywords: Chronic hepatitis C, Type 2 diabetes mellitus, Peginterferon and ribavirin.

INTRODUCTION

Chronic hepatitis C virus (CHCV) infection is gaining increasing attention as global health problem affects approximately 170 million people worldwide and long-term carriage may lead to the development of cirrhosis, liver decompensation and hepatocellular carcinoma, a major indication for liver transplantation [1]. Epidemiological studies indicate that each year approximately 3-4 million people worldwide are affected and>350000 individuals die due to liver disease [2]. There are 11 genotypes; genotypes 1-3 have a worldwide distribution accounting for 60-70 % of infections; type 3 predominantly seen in south - east Asia, type 4 restricted to Middle East and Africa. Genotypes 2 and 3 accounts for<30 %; whereas<1 % of total infection by genotype 5- 11 [3]. Egypt reports the highest prevalence of HCV worldwide, an average of 13.8 % with the predominant prevalence being infection with genotype 4a [4].

The link between chronic hepatitis caused by hepatitis C virus infection and type 2 diabetes mellitus has been suggested. Several studies have reported a higher prevalence of hepatitis induced by HCV in diabetic patients compared with control groups and other studies had found that there was a higher prevalence of diabetes mellitus in patients with chronic hepatitis C virus infection [5]. Additionally, type 2 diabetes (NIDDM) and insulin resistance (IR) are independent predictors of a more rapid progression of liver fibrosis and impaired response to antiviral treatment in chronic hepatitis C. In addition, IR and NIDDM not only accelerate the histological and clinical progression of chronic hepatitis C, but also reduce the early and sustained virological response (SVR) to interferon-alpha-based therapy [6]. As many as 80 % of patients with cirrhosis show glucose intolerance, and 10–20 % of them have diabetes mellitus [7]. Rapid virological response-undetectable HCV-RNA by polymerase chain reaction tests after the initial 4 w of treatment is an excellent positive predictor of SVR [8].

Interferon (IFN) induced thrombocytopenia and leucopenia is common, whereas anemia is more a sequel of combination therapy with ribavirin [9]. Moreover the main mechanism leading to hematological abnormalities during IFN therapy seems to be bone marrow suppression by IFN [10]. Although treatment-related side effects can make therapy unpleasant, most do not necessarily lead to discontinuation of therapy [11]. The goal of the present work is to determine the influence of type 2 diabetes mellitus on antiviral therapy with peginterferon alpha plus ribavirin in chronic hepatitis C patients.

MATERIALS AND METHODS

The participants in the study included 40 patients selected as out-patients at the hepatology division of Beni-Sueif general hospital**.** Permission from the authority of the hospital is to work protocol, is done and also every patient is accepting the investigations according to the medical ethics.

The patients were classified into two groups as the following:

Group I: included 20 patients with chronic hepatitis C with diabetes mellitus.

Group II: included 20 patients with chronic hepatitis C without diabetes mellitus.

Inclusion criteria

- Patients with chronic hepatitis C antibody positive and hepatitis B surface antigen negative.
- Patients with detectable HCV-RNA-PCR.
- Patients matching as regarding to age, viral load and the degree of inflammation and fibrosis.

- Patients with an established diagnosis of diabetes mellitus. Patients were diagnosed as having diabetes mellitus according to one of the classification criteria of the American Diabetes Association.

A-Random plasma glucose concentration ≥ 200 mg/dl (≥ 11.1 mmol/l), or

B-Fasting plasma glucose level ≥ 126 mg/dl (≥7.0 mmol/l). Or

C-Two hour's post-load plasma glucose level ≥ 200 mg/dl (≥ 11.1 mmol/l) during an oral glucose tolerance test [12].

Exclusion criteria

- Patients with other causes of chronic liver disease are excluded including hepatitis B virus infection (positive for hepatitis B surface antigen), drug-induced liver disease.
- Patient with advanced cirrhosis.
- Patient with hepatocellular carcinoma (HCC).
- Patients with serious complications in the heart, kidneys, or lungs.
- Patients with thyroid dysfunction.
- Patients with autoimmune diseases, such as autoimmune hepatitis.
- Patients with blood picture abnormalities such as anemia (hemoglobin concentration of 10 g/dl or less), leucopenia (white blood cells 1500/dl or less) and thrombocytopenia (platelets count 80.000/dl or less).

Treatment protocol

All patients were received peginterferon (Peg-IFN) and ribavirin (RBV) combination therapy. Peg-interferon-alpha 2b was given in weekly doses (1.5 µg/Kg/body weight). RBV was given in daily doses adjusted to body weight according to manufacturer's instructions (11 mg/kg/day). After 12 w of treatment, the virological response was recorded according to [13].

Sample collection

Venous blood sample collected from each subject before treatment was separated in a three tubes containing anti-coagulant (sodium florid) for measuring the fasting blood glucose or anti-coagulant (EDTA) for measuring the hematological parameters. The blood in the last tube allowed to clotting then centrifuged to separate the serum, for the other tests. And all tests were done in Beni-Suief general hospital. The samples were collected prior the starting of treatment to determine the recommended baseline laboratory tests included in the treatment approval form of National Hepatology for Tropical Medicine and Research Institute (TMRI) for patients with chronic HCV infection who are being considered for antiviral therapy. Chronic hepatitis C patients who had contraindications to interferon can't be treated with the antiviral combination therapy. After 12 w of treatment another blood samples was collected from patients to evaluate the virological, biochemical and hematological responses.

Parameters investigated before and after treatment

Viral load

Hepatitis C virus RNA (HCV-RNA), (REAL TIME PCR)

Hepatitis C virus RNA was carried out by using nested PCR, which was performed parallel with positive and negative controls on light cycler Real-Time and on–line quantification using Roche Amplicor HCV monitor version 2.0 (Roche Diagnostics, Branchburg, NJ) for measurements. The low limit of detection was 50 IU/ml.

Biochemical parameters

Estimation of serum aminotransferases (ALT), (AST)

Determination was done according to [14] using Kits of noble diagnostic, Egypt. Chemical analyzer (HERA, Linear chemicals, S. L. Spain) was used for measuring biochemical parameters.

Determination of serum bilirubin

The test performed according to [15] using kits of diamond diagnostic, Egypt.

Hematological parameters

The hematological parameters include measurement of hemoglobin concentration, white blood cell count and platelets count according to [16] using a cell counter instrument (HeCo SEAC).

Parameters investigated before starting the treatment

The following parameters were investigated according to the approval form of National Hepatology for Tropical Medicine and Research Institute (TMRI), as a pre-enrollment data for patients who are being considered for antiviral therapy.

Hepatitis C virus antibodies (anti–HCV)

Hepatitis C virus antibodies were performed with a third–generation ELISA according to [17] using the instrument READER, 2000 and HCV BIO kits S. A Barcelona.

Hepatitis B surface antigen (HBs Ag)

Hepatitis B surface antigen was performed by enzyme linked immunosorbant assay according to [18] using the instrument Radium, Germany.

Serum albumin

The test was performed according to [19] using kits of Diamond Diagnostic, Egypt.

Serum alkaline phosphatase (ALP)

Estimation was done according to [14] using kits of Bio System, Spain.

International normalized ratio (INR)

The test was performed according to [20] using kits of Dia-Med, Switzerland.

Fasting plasma glucose

Estimation was done according to [15], using kits purchased from Diamond Diagnostic, Egypt.

Serum creatinine

The test was investigated according to [21] using kits purchased from Diamond Diagnostic, Egypt.

Serum alphafetoprotien (AFP)

Determination was done according to (22) using kits of VIDAS bio-Merieux, France and Mini VIDAS instrument.

Thyroid stimulating hormone (TSH)

The test was done using kits of VIDAS bio-Merieux, France. The assay principle combines a one–step enzyme immunoassay sandwich method with a final fluorescent detection ELFA (Enzyme Linked Fluorescent Assay) according to [23]. The test was done using Mini VIDAS instrument.

Anti-nuclear antibody titer (ANA)

The parameter was investigated according to [24] using kits of Bio-Quant-U S and the automated ELECSYS 1010, Roche instrument.

Anti bilharzial antibodies

Determination was done according to [25] using Kits of FUMOUZE diagnostic, France. The principle based on indirect hemoagglutination.

Ultrasonographic guided needle liver biopsy

Percutaneous needle biopsy was performed under ultrasound guidance because of lower rate of complication [26]. A satisfactory biopsy is 1-4 cm long. In general a biopsy of 1.5 cm with four to six

portal tracts is sufficient for histological assessment of chronic hepatitis. The liver biopsies were scored using the Ishak modified HAI [27].

Ethical aspects

- All patients have given a written consent as regard the participation in the study and having the right to withdraw from the study, according to an ethics committee of hepatology unit, in Beni-Sueif general hospital.
- All the selected patients have given a written consent as regard to liver biopsy.
- For the matter of confidentiality, the clinical assessment and transformation of data were done by the researcher.

Statistical analysis of the results

Data was collected coded and analyzed using SPSS software version 17 under Windows Vista. Descriptive analysis of the results was done in the form of percentage distribution for qualitative data, and mean and standard deviation calculation for quantitative data. It was followed by applying the suitable tests of significance for the comparison between different groups and 95 % level of significance is considered (SPSS version 17 SPSS INC., Chicago, IL, USA). The following tests of significance were used: Fisher's Exact Test, Student t-test and Paired t-test. The results were expressed as the mean±standard deviation (SD) and values of P>0.05 were considered statistically non-significant (N. S.), while values of P value<0.05, 0.01, 0.001 were considered statistically significant (Sig).

RESULTS

Baseline pretreatment assessment included complete blood picture, liver and kidney functions, quantitative HCV-PCR, TSH levels, the liver biopsy while, Liver functions, CBCs and quantitative HCV–PCR and followed up after 12 w of treatment.

Baseline parameters before treatment

- **Demographic distribution**

In the study, the patient's demographic distribution was as follows:

There were no significant differences in the age or sex between the two groups p value (>0.05) as shown in table (1).

- **Laboratory investigation**

Patients included in the study, screened for hepatitis C virus antibodies were positive for HCV abs. and all patients were negative for hepatitis B surface antigen.

As shown in table (1), the base line data collected from both groups before treatment indicated that there was a significant difference (p value<0.05) regarding fasting blood glucose and a non-significant statistical difference (p value>0.05) was found regarding sex, age, serum creatinine, serum albumin, serum alkaline phosphatase, serum AST, serum ALT, total bilirubin, indirect bilirubin, total leucocytes count, hemoglobin concentration, platelets count, INR, ANA titer, TSH, alpha-fetoprotein, viral load and the degree of cirrhosis. The two groups before treatment have the same baseline of biochemical and hematological features, and also the same degree of cirrhosis and viral load and all patients had no contraindication for antiviral combination therapy.

Study of the variable responses of both groups after treatment

Effect of antiviral treatment on biochemical parameters

- **Effect on serum glutamate pyruvate transaminase activity (ALT)**

In our study, a normalization of serum alanine transaminase was observed after 12 w of antiviral combination therapy. The decrease in ALT activity of group one after treatment as compared to ALT activity before treatment was highly significant as shown in table (2). Also, group two showed a highly significant decrease in ALT activity after treatment as compared to their activity before treatment as represented in table (3).

- **Effect on serum glutamate oxaloacetate transaminase activity (AST)**

A normalization of AST was observed after 12 w of antiviral combination therapy. The decrease in AST activity was highly significant in group I after treatment as compared to AST activity before treatment, as represented in table (2). Group II also showed a highly significant decrease in AST activity after treatment as compared to AST activity before treatment, which observed in table (3).

- **Effect on total bilirubin**

In group I bilirubin concentration showed a significant decrease (<0.001) after treatment as compared with before treatment (table 2). Furthermore, group II showed a significant decrease in total bilirubin after treatment as compared to before treatment (table 3).

Effect of antiviral treatment on viral load

Table 4 shows the evaluated measurements of serum HCV RNA as a predictor of response 12 w after starting antiviral therapy. Group II revealed a clearance to be of 95 % while in group I the clearance with 65 % significantly lower (P<0.05). Furthermore, patients with lower pre-treatment HCV-RNA concentration had a high likelihood of achieving a sustained response.

Effect of antiviral treatment on hematological parameters

- **Effect on hemoglobin**

In the present study, a significant decrease (<0.001) in hemoglobin concentration after treatment in group I was detected as compared to that before treatment (table4). Anemia with hemoglobin (Hb) levels below 10 gm was detected in 5 cases (32 %) after weeks 12 of treatment. In group II, also indicated a significant decrease in hemoglobin concentration after treatment compared with that before treatment (table 5). 4 cases (16 %) showed hemoglobin less than 10 mg/dl, while 1 case showed marked anemia and forcing the discontinuation of treatment.

- **Effect on platelets**

Patients with chronic hepatitis C showed after treatment with peginterferon and ribavirin a significant decrease (<0.001) in peripheral platelets compared to that before treatment in group I as shown in table (4). However, in 15 % of cases platelet count was less than 120.000 cells/ml^3. Group II, showed also a significant decrease in peripheral platelets after treatment compared to that before treatment (table 5). On an other hand, in 25 % of all cases there was platelet count less than 120.000 cell/ml^3.

- **Effect on leucocytes**

Treatment with peginterferon and ribavirin decreased in group one white blood cells counts significantly (<0.001) as represented in table (4). 28 % of cases showed leucocytes count less than 3000 cell/ml^3**.** Group II revealed also, a significant decrease in white blood cells count after treatment as compared to before treatment as illustrated in table (5).

Comparison between the two groups after treatment

As shown in table (6), there were significant differences (p value<0.05) between group I and II concerning various parameters. The mean values of ALT, total bilirubin and WBCs were significantly higher in group I than in group II. There were no significant differences between groups in AST, hemoglobin and platelets count.

DISCUSSION

HCV genotype 4 is prevalent in Egypt which is responsible for more than 90 % of infection and is considered as a major cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) and liver transplantation [28]. The current treatment for patients with chronic hepatitis C genotype 4 is a combination therapy using subcutaneous peg-interferon (PEG-IFN), together with oral ribavirin

(RBV) for 24 to 48 w. Moreover, 12 w of antiviral therapy HCV-RNA should be tested to determine the viral response. If the HCV-RNA level at 12 w has decreased to less than 2 log10 IU/ml compared with the pretreatment HCV-RNA level, it is advised to stop treatment because an SVR will rarely occur [29]. The effectiveness of antiviral treatment, the extent to which treatment can clear the viral infection is assessed according to the proportion of patients achieving sustained virologic response (SVR). SVR is the fundamental goal of treatment and is defined as undetectable (or below the lower limit of quantification) HCV RNA at 12–24 w after cessation of treatment [30]. A link between chronic hepatitis caused by chronic hepatitis C infection and type2 DM has recently been suggested. Several studies have reported a higher prevalence of hepatitis induced by HCV in diabetic patients compared with control groups. Others had found the higher prevalence of DM in patients with chronic HCV infection [5, 31].

Table 1: The base lines of several parameters of group 1 as compared to group 11 before treatment

Base line data	Group I	Group II
	mean±SD	mean±SD
Age	46.25±4.2	45.20±5.2
Glucose (mg/dl)	152.5±19.8**	83.65±10.7
Creatinine(mg/dl)	0.88±0.16	0.84±0.14
Albumin(g/dl)	3.89±0.18	3.94±0.30
Alkaline ph(U/l)	140.60±27.5	138.30±24.0
AST (U/l)	79.00±10.9	72.60±12.2
ALT (U/l)	99.00±15.1	92.05±10.9
Total bilirubin(mg/dl)	1.3±0.21	1.23±0.20
direct bilirubin(mg/dl)	0.46±0.13	0.43±0.11
INR	1.26±0.08	1.23±0.14
Alpha-fetoprotein	9.0±1.1	8.5±1.06
TSH	1.7±0.36	1.9±0.47
WBCs(cell/ml^3)	6074±1161	6047±1030
HB (g/dl)	14.12±0.87	13.34±1.47
PLT (cell/ml^3)	209300±19431	215900±24065
HCVRNA x10^3(IU/ml)	655.457±121.158	508.638±101.732
Degree of cirrhosis	1.85±0.34	1.70±0.30

Data expressed as mean and±SD. Number of patients in each group were twenty. P*<0.05, p**<0.01, P<0.001

Group I: patients with chronic hepatitis C with diabetes mellitus, **Group II**: patients with chronic hepatitis C without diabetes mellitus.

Table 2: Effects of treatment on some liver function parameters of Group I

Parameter	Group I before treatment (control)	Group I after treatment
	mean±SD	mean±SD
ALT(U/l)	99.0±15.13	27.05±6.67**
AST (U/l)	79.0±10.95	30.20±8.97**
T bilirubin (mg/dl)	1.3±0.21	0.83±0.16**

Data expressed as Mean and±SD. Number of patients in each group was twenty. P*<0.05, p**<0.01, P<0.001

Table 3: Effects of treatment on some liver function parameters of Group 1I

Parameter	Group II before treatment (control)	Group II after treatment
	mean±SD	mean±SD
ALT (U/l)	92.05±10.99	23.50±6.35**
AST (U/l)	72.60±12.22	24.95±3.84**
T bilirubin (mg/dl)	1.23±0.2	0.70±0. 12**

Data expressed as Mean and±SD. Number of patients in each group was twenty. P*<0.05, p**<0.01, P<0.001

Table 4: Hematological results of Group I before and after treatment

Parameter	Group I before treatment (control)	Group I after treatment
	mean±SD	mean±SD
WBCs(cell/ml^3)	6074±1161	3232±590**
HB(mg/dl)	14.12±0.87	10.19±1.07**
Platelets(cell/ml^3)	209300±19431	156300±28394**

Data expressed as Mean±SD Number of patients in each group was twenty. P*<0.05, p**<0.01, P<0.001.

Table 5: Hematological results of Group II before and after treatment

Parameter	Group II before treatment (control)	Group II after treatment
	mean±SD	mean±SD
WBCs(cell/ml^3)	6047±1230	3690±415**
HB(mg/dl)	13.34±1.47	10.86±1.33**
Platelets(cell/ml^3)	215900±24065	168500±28162**

Data expressed as Mean and±SD. Number of patients in each group was twenty. P*<0.05, p**<0.01, P<0.001

Table 6: Comparison of mean rank of change of group 11 as compared to group 1 parameters after treatment

Parameter	Group I after	Group II after
	mean±SD	mean±SD
ALT(U/l)	30.20±8.97	23.50±6.35*
AST(U/l)	27.05±6.67	24.95±3.84
Total bilirubin(mg/dl)	0.83±0.16	0.76±0.12*
WBCs(cell/ml^3)	3232±590	3690±415*
HB (mg/dl)	10.19±1.07	10.86±1.33*
Platelets (cell/ml^3)	156300±28394	168500±28162

Data expressed as mean and±SD. Number of patients in each group were twenty. P*<0.05, p**<0.01, P<0.001

Data of the present study indicated that there were a non-significant difference between both groups in alkaline phosphatase (ALP) activity and ALP activity was within normal ranges in most cases. Furthermore, non-significant differences between both groups were observed in total bilirubin, direct bilirubin, albumin concentrations and international normalized ratio (INR). These results are consistent with results of [32] who reported that alkaline phosphatase; total and direct bilirubin, albumin concentration and INR are usually within normal ranges in chronic hepatitis C patients. The author data indicated that elevation of ALP; total and direct bilirubin and INR may indicate cirrhosis of liver cells [32]. Albumin levels, bilirubin, and pro-thrombin time are normal until late-stage disease [33].

Present data indicated that there was a non-significant difference in alpha fetoprotein (AFP) between both groups, and both groups showed a normal value in comparing to normal ranges. This indicated that all patients have no evidence of hepatocellular carcinoma (HCC). Also, there was a non-significant difference in thyroid stimulating hormone (TSH) and anti-nuclear antibodies values in both groups. Also, there was non-significant change in creatinine concentration between both groups. And levels of AFP, TSH, ANA and creatinine were within normal ranges and not contra indicated to antiviral treatment.

The current investigation showed a significant increase in ALT ranging from 1 to 3 times of the upper limit of normal reference ranges in group one and group two. There was non-significant difference between both groups in ALT activity. Also, there were a significant increases in AST range from 1 to 2 times of the upper limit of normal ranges in group one and group two, and non-significant difference between both groups in AST activity. Moreover, serum ALT levels are usually higher than AST levels. These data agree with [32] who reported that increases in the ALT and AST range from zero to 20 times (but usually are less than five times) of the upper limit of normal in CHC but that finding may be reversed in patients who have cirrhosis [33]. Chronic infection causes mild chronic inflammation of the liver and leakage of liver enzymes, ongoing cycles of inflammation, necrosis and apoptosis eventually leading to scarring (fibrosis) and, ultimately, severe bridging fibrosis with nodular regeneration [34]. Above data showed non-significant changes between groups in biochemical baseline tests in between both groups.

In the present study, after 12 w of antiviral combination therapy a highly significant decrease in ALT and AST activities was observed in both groups after treatment as compared to their activity before treatment. As transaminases, bilirubin concentration showed also a highly significant decrease after treatment as compared with before treatment in both groups

Our findings are in accordance with [35], who reported that a virological response to peg-interferon and ribavirin is typically associated with a decrease in serum transaminases. Also, [36] found that the decline rates of ALT from baseline to week 2 and 4 of INF and RBV combination therapy are good predictors of an SVR. Also, [37] studied one hundred seven patients with chronic Hepatitis C (genotype 1), and recorded that all liver tests return to normal with eradication of the Hepatitis C virus. When comparing both groups with each other after treatment in response to antiviral treatment, the presented data showed that there were significant increases in the mean change of ALT activity and total bilirubin in group one in comparing with group two. However, there were a non-significant difference in the mean change of AST activity during the course of treatment in group one as compared to group two after treatment. The results of this investigation is in accordance with the results of [35] who reported that transaminases elevations were associated with greater pre-treatment body weight, insulin resistance, and poorer sustained virological response rates. Since HCV promotes insulin resistance and insulin resistance induce fibrosis and interferon resistance resulting in poor virological response [38].

Transaminases elevations during treatment of chronic hepatitis C virus with peg-interferon and ribavirin are common but rarely severe in the present study there were 4 cases of group one showed increase in ALT activity. This mild rises may reflect ongoing viral activity in treatment non-responders. Highly significant increase is frequently observed despite a virological response, and may be because of an immuno-modulating effect of interferon in susceptible patients [35]. These findings are in accordance with [39], who reported that Serum ALT level became normal by the end of treatment in 80 of the 110 patients (73 percent).

Hepatitis C Virus (HCV) infection appears to confer increased risk of diabetes mellitus beyond established risk factors and is associated with severity of liver disease [40]. Delayed biochemical response was associated with the baseline hepatic fibrosis stage and liver cirrhosis [41]. The present data demonstrated that the biochemical response to antiviral therapy in group two was better than group one. This agrees with [6] who reported that T2DM accelerate the histological and clinical progression of chronic hepatitis C and reduce the response to therapy in CHC patients. From the above mentioned results, after 12 w of antiviral therapy a normalization of liver tests occurred, and diabetic group showed a delayed biochemical response.

In the presented study, there were non-significant changes in white blood cells (WBCs) count; hemoglobin concentration and platelets count between both groups before starting the antiviral therapy and all studied patients have no evidence of anemia, leucopenia and thrombocytopenia. These base line parameters are in agree with [39] who showed how to evaluate the efficacy of combination of standard interferon α2b and ribavirin in chronic hepatitis C. All included 126 patients showed normal base line hematological parameters. Side effects of treatment however are essentially universal. These led to modification of the dosage of interferon and/or ribavirin in 35-42 % of patients treated with peg-interferon, in large randomized clinical trials and discontinuation of therapy in 14-19 % of these patients [42]. Patients were defined as having hematological abnormalities if they had presence of anemia, neutropenia, thrombocytopenia, or a combination of the above during treatment with interferon and ribavirin [43].

The most frequent hematological abnormalities including anemia, thrombocytopenia, and leucopenia are commonly seen in patients with chronic hepatitis C treated with combined antiviral treatment [44]. In the present study, a highly significant decrease in hemoglobin level was observed in group one after treatment as compared to before treatment. Anemia with hemoglobin levels below 10 gm was detected in 25 % of cases at week 12. A modification of treatment occurred and 10 % of cases show marked anemia with hemoglobin level ≤8.0g/dl which led to the discontinuance of treatment. In group two, we found a highly significance decrease in hemoglobin concentration after treatment.

There were 4 cases (20 %) showing hemoglobin less than 10 mg/dl and 1 case (5 %) showed marked anemia forcing the discontinuance of treatment. Furthermore, this study demonstrates that 30 % of the studied patients (12/40) had decrease in Hb below 10 gm, 7 cases of group one and 5 cases of group two. These results of the current study are in agreement with those of [42] who found that>20 % of patients treated with a peg-interferon and ribavirin at 1,000-1,200 mg/day had marked anemia. Because ribavirin-induced hemolysis destroys a portion of the available pool of erythrocytes, a high pre-treatment level of hemoglobin was the logical explanation for its large decrease. Erythrocytes selectively accumulate RBV metabolites, sustained oxidative membrane damage and become subject to increased extra vascular hemolysis in the reticuloendothelial system. In addition, the IFNs can directly suppress bone marrow erythropoiesis. Ribavirin itself has also been reported to have myelosuppressive properties [45]. Furthermore, [39] found that the mean hemoglobin concentration fell from 13.2±1.2 g per deciliter to 11.2±1.6 g per deciliter during the first month of treatment. The values fell below 10 g per deciliter in 10 patients and below 9 g per deciliter in 3 patients.

As regarding the total leucocytes count, the present data demonstrated that treatment with peg-interferon and ribavirin showed a highly significant decrease in white blood cells count after treatment as compared to before treatment in group one and group two. The present results revealed the incidence of leucopenia with decreased WBCs (3000 cell/ml^3) in 15 % of cases after 12 w of therapy. These data are consistent with [42] who found that approximately 20 % of treated patients had neutropenia. Also, [45] noted that patients receiving PEG-IFN alpha-2b and RBV have dose modifications for neutropenia in 18 % of patients receiving 1.5 mcg/kg of PEG-IFN alpha-2b. Also, [39] found that white blood cells decreased by 35 % and neutrophil count were reduced by 25 % after 4 w of treatment.

The present result indicated that there were significant decreases in platelets count during interferon and ribavirin therapy. Patients that had platelets count below 120.000 cell/m^3 during the course of treatment was 25 % of group one and 15 % of group two. Also, obtained results demonstrated that there was non-significant difference in the mean change of platelets in comparing diabetic and non-diabetic patients after treatment.

Moreover, [46] found that only around 4-6 % of patients receiving PEG-IFN alpha-2a and RBV required dose reductions for thrombocytopenia. This was broadly comparable with patients treated with the PEG-IFN alpha-2b/RBV combination (3 %) and standard IFN/RBV (1 %). Additionally, [45] reported that interferon therapy frequently results in a 10-50 % fall in the platelet count. Moreover, [39] recorded that the platelet counts as a biological response also fell by 7 % only in five patients. Possible mechanisms include a relative thrombopoietin deficiency, impaired thrombopoietin signal transduction in megakaryocytes and in some cases increased immune-mediated sequestration of platelets. Also, the high incidence of thrombocytopenia observed in the two previous studies may be attributed to association with hypersplenism; low baseline platelets count [46].

Furthermore, [43] studied the hematological abnormalities during treatment with interferon and ribavirin in 136 patients with chronic hepatitis C showed 52 (38.2 %) of the patients developed significant hematological abnormalities with 28 (20.6 %), 30 (22.1 %), and11 (8.1 %) developed neutropenia, anemia, thrombocytopenia.

The present study demonstrated that there were significant difference in the mean change of hemoglobin concentration in group one as compared to group two, and a highly significant difference in WBCs in group one as compared to group two after combination of interferon and ribavirin therapy. From above mentioned results, data demonstrated that the hematological abnormalities during therapy were more frequent in diabetic compared to non-diabetic HCV patients.

In the present study measurement of serum HCV-RNA after 12 w of starting antiviral therapy used as a predictor of response, which proved that clearance of serum HCV-RNA was more likely in patients with lower pre-treatment HCV RNA concentration and they had a high likelihood of achieving a sustained response. It was clear from the presented data that in group one diabetic CHC patients, 7 non responder patients showed 2 patients with moderate and 5 patients with high viremia. On the other hand, in group two non-diabetic CHC patients, the only non-responder case was with high viremia. These results are in agreement with [47] who reported that high baseline viral load means that the virus is reproducing rapidly and viral cure/SVR may be less likely to occur. Patients with a significant decrease in HCV-RNA may demonstrate decreased serum liver enzyme, e. g., alanine aminotransferase (ALT) levels and reduced liver tissue damage [48]. Additionally, (49) reported that a higher HCV RNA level predicts a lower response rate. The impact of HCV RNA level on the response to combination therapy was different between patients with different HCV genotype infections. High viral load influenced the response rate in patients with HCV-1 (41 % versus 56 %) but not those in patients with HCV-2 or HCV-3 (74 % versus 81 %). Above mentioned results showed that clearance of serum HCV RNA was more likely in patients with lower pre-treatment HCV-RNA concentration. The present study revealed that there was a significant decrease in diabetic response when compared to non-diabetic patients to antiviral combination therapy with a sustained virological response (SVR) in 65.0 % of patients with CHC and type 2 diabetes mellitus and on the other hand 95 % of CHC patients without diabetes mellitus showed SVR. Furthermore, [38] found that sustained virological response (SVR) occurred in 23 of 70 (32.8 %) of patients with genotype 1 and insulin resistance, while in 26 of 43 (60.5 %) of genotype 1 patient without insulin resistance. These findings were independently extended to non-responders with genotypes 2 and 3 [50]. The present results are consistent with the results of [51] who study the influence of DM on the outcome of INF-alpha2b plus ribavirin therapy. In a cohort of 110 patients with CHC, the outcome therapy was evaluated by comparing the patients with and without DM. There were 46 sustained–responders while 64 patients did not become sustained responders which indicated that DM reduces the response to INF-alpha plus ribavirin therapy in CHC patients of genotype1.

Combination therapy has brought their rate of long-term viral clearance and significantly advanced the treatment of chronic hepatitis C and represents the current standard of care [52]. An SVR is the optimal outcome of HCV therapy. Liver inflammation improves and the rate of fibrosis progression is slowed [53]. A 2-10 folds increase in type 2 DM has been reported worldwide in HCV-positive patients when compared with patients with other forms of liver diseases [54]. From the clinical standpoint, insulin resistance accelerates fibrogenesis and impairs response to IFN-α-based antiviral therapy [38]. Moreover [55], showed that in a study of 1059 patients with chronic HCV the sustained virological response (SVR) rate was lower in patients with impaired fasting glucose (IFG) and/or T2DM than in patients with normal glucose concentrations.

Additionally, [56] reported that IR and type 2diabetes (T2DM) not only accelerate the histological and clinical progression of chronic hepatitis C, but also reduce the early and sustained virological response to antiviral therapy. Furthermore, [57] reported that glycemic control in chronic hepatitis C, could improve the prognosis and the response to antiviral treatment. From the above mentioned studies, type 2 DM was associated with impaired virological response to Peg-INF/RBV in CHC patients.

In conclusion, the present study has shown that type 2 diabetes mellitus in CHC predicts a poor response to antiviral therapy and predict faster progression to fibrosis and cirrhosis that may be culminates in liver failure and hepatocellular carcinoma. The delayed biochemical response during therapy was more frequent in diabetic compared to non-diabetic CHC patients.

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CONFLICT OF INTERESTS

The authors declare that we have no conflict of interest.

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