Association of ITPA Gene Expression and Hematological Abnormalities Induced by Dual therapy in HCV-infected Patients

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Abstract

The combination therapy has been the standard treatment for chronic hepatitis C virus (HCV) infection, but many patients have side effects due to this therapy. Recently, new direct-acting antivirals (DAAs) have enhanced the response rate, especially in difficult-to-treat patients infected with HCV genotypes (GT) 1 or 4, and have made adverse effects less common. RBV plays continually an important role in HCV therapy even with the introduction of DAAs. Hemolytic anemia is a common side effect in HCV-infected patients on combination therapy, affecting up to 30 % of patients, which needs to followup hemoglobin and dose modification in up to 15 % of patients. RBV-induced anemia primarily results in the reduction of adenosine triphosphate (ATP) levels in erythrocytes, affecting ATP-dependent oxidative metabolism. Genetic variations in the inosine triphosphatase (ITPA) gene, which encodes an inosine triphosphate pyrophosphohydrolase (ITPase), protect from hemolytic anemia during dual therapy. These ITPA variants affect ITPase functionality, causing a drop in its activity, resulting in an accumulation of inosine triphosphate (ITP) in erythrocytes and the prevention of oxidative stress. The functional variants in ITPA gene leads to ITPase deficiency and a strong accumulation of ITP in erythrocytes, which is associated with lower RBV-toxicity. These genetic variants have been associated with protection from anemia.

Keywords: ITPA, Hemolytic anemia, Ribavirin, Chronic hepatitis C, HCV therapy.

Introduction

Hepatitis C virus (HCV) infection has a worldwide prevalence of approximately 2.2–3.0% affecting approximately 130 to 170 million people worldwide (**Lavanchy, 2009**). The treatment success with ribavirin therapy, defined as sustained virological response (SVR), has been reported to be between 54% and 63% in clinical trial settings (**Manns *et al*., 2001; Fried, 2002 and McHutchison *et al*., 2007**). Hematological abnormalities including

neutropenia, anemia, and thrombocytopenia are commonly seen in patients with chronic hepatitis C treated with ribavirin therapy (**Jagdish** *et al*., **2010).** Recent studies indicate that the combination therapy which contained ribavirin is effective for chronic hepatitis C virus (HCV) infection. However, reversible hemolytic anemia is a common side effect of this therapy (**Shintaro** *et al*., **2004**). Hematological abnormalities were based on the dosage reduction guidelines in the package inserts for ribavirin. Anemia was defined as hemoglobin less than 10 gm/dl, neutropenia was defined as an absolute neutrophil count less than 750 cells/mm^3, and thrombocytopenia was defined as a platelet count less than 50,000 cells/mm^3 (**Jagdish** *et al*., **2010)**. Adherence and optimal dosing positively influence SVR rates (**McHutchison** *et al*., **2002; Mulhall** *et al*., **2005 and Reau** *et al*., **2008**). Adherence is decreased by side effects such as multiple hematological abnormalities including neutropenia, anemia, and thrombocytopenia. The side effects can be managed by reducing the doses of ribavirin but suboptimal doses negatively affect treatment outcomes. Timing of growth factors such as erythropoietin in the early stages of hematological abnormalities can ensure patient compliance while maintaining or improving patient quality of life (**McHutchison** *et al*., **2007 and Reau** *et al*., **2008**). But these growth factors take several weeks to attain full potential; hence, early detection of hematological abnormality becomes very important. If we can identify patients who are at risk of developing hematological abnormalities before start of therapy, these patients can be monitored more aggressively, and timely decisions for additional interventions such as growth factors can be made. Such interventions can improve patient outcomes. One of the most common hematological abnormalities associated with the use of ribavirin and chronic hepatitis C (CHC) infection is the development of dose-dependent hemolytic anemia (**McHutchison** *et al*., **2007**). Other hematological abnormalities associated with the use of ribavirin are the development of neutropenia and thrombocytopenia**. (Ong** *et al*., **2004 and Kowdley, 2005**). Inosine triphosphatase (ITPA) gene, located on chromosome 20, is involved in the protection against RBV-induced hemolytic anemia. Genetic polymorphisms in

ITPA gene were associated with two functional variants that resulted in ITPase deficiency with subsequent prevention of depletion of erythrocyte adenosine triphosphate (ATP), oxidative damage to the erythrocyte membrane (**Hitomi** *et al*., **2011**) and eventual protection against RBV-induced hemolytic anemia (**Fellay** *et al*., **2010**). The highest reduction in ITPA activity was due to mutation in exon 2 (94C>A), where heterozygotes (CA) had 22.5% residual activity and homozygotes (AA) had zero activity (**Sumi** *et al*., **2002**). ITPA hydrolyzes inosine triphosphate (ITP) and deoxy inosine triphosphate. Deficiency or reduced function of the ITPA protein leads to accumulation of ITP. ITP can substitute for guanosine triphosphate (GTP) (which is depleted by RBV) as a substrate for adenylosuccinate synthase to produce adenylosuccinate, which can be further converted into ATP. It has been shown that RBV reduces erythrocyte ATP levels. Thus, in ITPA deficient patients, ITP accumulates in the erythrocytes and helps restore ATP levels, preventing lysis due to loss of ATP (**Hitomi** *et al*., **2011**). Inosine triphosphatase (ITPA) gene variants can protect against ribavirin (RBV)-induced anemia in patients treated for chronic hepatitis C. The genetic variation in Inosine Triphosphate Pyrophosphatase (ITPA) encoding inosine triphosphatase (ITPase) was a protective factor against hemolytic anemia in patients receiving RBV therapy (**Fellay** *et al*., **2010 and Thompson** *et al*., **2011**). The ITPase catalyses the conversion of inosine triphosphate (ITP) to inosine monophosphate (IMP), and pyrophosphate, so the ITP does not accumulate in normal cells. There are two single nucleotide polymorphisms (SNPs) in ITPA gene, located on chromosome 20 (**Simone** *et al*., **2013**). These two ITPA polymorphisms are associated with reduced ITPase activity resulting in accumulation of ITP in red blood cells (RBCs) and subsequent hemolysis (**Von** *et al*., **2008 and Tanaka** *et al*., **2012**). RBV can be incorporated into erythrocytes, where it undergoes phosphorylation to its active forms through adenosine kinase. The RBV triphosphate conjugates cannot cross the erythrocyte cell membrane and accumulate in the intracellular compartment, causing oxidative damage and leading to haemolysis (**Kuehn, 2014**). RBV causes the depletion of ATP which is followed by guanosine triphosphate (GTP) deficiency in RBCs and causes RBC lysis (**De Franceschi** *et al*., **2000**).

Accumulated ITP instead of RBV triphosphate in ITPase-deficient patients would play a great role for compensating the depletion of ATP, and prevention of oxidative reactions and RBC lysis (**Thompson *et al*., 2011**). The ITPase deficiency causes accumulation of ITP in RBCs that may compete with RBV triphosphate, thereby protecting from RBV-induced haemolysis (**Sakamoto *et al*., 2010; Hitomi *et al*., 2011; Kim *et al*., 2013 and Peltenburg *et al*., 2015**). Although ITPase deficiency is protective against anemia, it has been associated with a greater decline in platelet count during RBV therapy (**Rembeck *et al*., 2014**). In agarose gel electrophoresis, Agarose is usually used in gel between 0.7% and 3%, which determines the pore size. Lower concentrations result in larger pore sizes, whereas higher concentrations result in smaller pore sizes. Agarose gels are generally used to separate larger nucleic acid molecules such as DNA or RNA because the pore sizes are large enough for these molecules to pass through the gel. (**Ashok *et al*., 2017**).

The aim of this study was to investigate the association between inosine triphosphatase (ITPase) activity and the degree of anemia occurring during ribavirin theraby in hepatitis C virus (HCV)-infected patients.

Materials and Methods

Collection of samples:

Serum, plasma or whole blood samples were prepared by collecting a blood sample from the vein. Blood was collected in serum separation tube. Samples were allowed standing for 15 to 30 min at room temperature, and then centrifuged at 3000 rpm for 10 min. The serum was harvested by a micropipette into an Eppendorf tubes and stored at – 20º C for assay later on the same day. Blood samples were collected in heparinized tube, mixed gently without shaking, and then centrifuged at 3000 rpm for 10 min. Plasma samples were then separated by a micropipette into an eppendorf tubes and stored at – 20º C for further analysis. Blood samples were collected in heparinized or EDTA tubes; mixed gently with gentle shaking to mix blood with anti- clotting factor. The predicted inosine triphosphate pyrophosphatase (ITPase) activity was studied. Serum HCV RNA determined using a qualitative polymerase chain reaction (PCR) assay (Amplicor HCV version 2.0, Roche Molecular Systems, Pleasanton, CA, USA). Liver function tests, CBC, Fasting blood sugar and INR were measured by the automated Cobas C311. TSH, AFP and HBsAg were measured by the automated Cobas A411. Two milliliters of blood were put into vaccutainers containing ethylene diamine tetra-acetic acid (EDTA) for DNA extraction and analysis of ITPA polymorphism using Real time PCR-assay. Total genomic DNA of patients was extracted using Qiagen extraction kit. Rotor-Gene 6000 (QIAGEN, ABI system, USA).

RNA extraction Procedure:

Centrifuge 50-500 µL of blood for 5 min then Resuspend the pellet in 600 µL of Lysis Buffer, mix well by vortexing. Add 450 µL of ethanol (96-100%) and mix well. Transfer half of the prepared lysate to a column inserted in a collection tube. Centrifuge the column for 1 min at 12,000 × g (~11,000 rpm). Discard the flow through solution and reassemble the column and collection tube. Transfer remaining lysate into the column and centrifuge as before. Discard the collection tube containing the flow-through solution. Place the column into a new 2 mL collection tube (included). Add 700 µL of Wash Buffer WB 1 (with ethanol added). Centrifuge for 1 min at 12,000 × g (~11,000 rpm). Discard the flow-through and place the purification column back into the collection tube. Add 500 µL of Wash Buffer 2 (with ethanol added) to the purification column. Centrifuge for 1 min at 12,000 × g (~11,000 rpm). Add 500 µL of Wash Buffer 2 (with ethanol added) to the purification column. Centrifuge for 2 min at 12,000 × g (~11,000 rpm). Empty the collection tube. Place the purification column back into the tube and re-spin the column for 1 min at maximum speed (≥20,000 × g, ≥14,000 rpm). Discard the collection tube containing the flow-through solution and transfer the purification column to an RNase-free 1.5 mL micro centrifuge tube. Add 50 µL of nuclease-free water to the center of the purification column membrane and centrifuge for 1 min at 12,000 × g (~11,000 rpm). Discard the purification column. Use the purified RNA immediately in downstream applications or store at -20°C until use. Keep the RNA on ice after extraction and while working with it.

Generation of cDNA Procedures:

The first-strand cDNA was generated for use in two-step RT-PCR. Add into sterile, nuclease-free tube on ice 5 µg of Template RNA, 0.2 µg Primer and complete to 12.5 µL DEPC-treated water then centrifuge and place on ice. Add 4 µL buffer, 0.5 µL RNase Inhibitor, 2µL dNTP, 1 µL Reverse Transcriptase then Mix gently and centrifuge. **(Gerard and D'Alessio, 1993)**.

Real-time PCR:

Maxima SYBR Green qPCR Master Mix (2X), ROX Solution provided was used. Primer design for qPCR is one of the most important factors to obtain efficient amplification and to avoid the formation of primer dimers. Sequences of the primers which were used in the reaction are Forward (TCA TTG GTG GGG AAG AAG ATC) and Reverse (AAG CTG CCA AAC TGC CAA A) **(Longo *et al.*, 1990)**.

Detection of PCR products:

PCR products are generally detected by agarose gel electrophoresis using Fifteen μl of amplified product and mixed with 4 μl of loading buffer (0.1% bromophenol blue, 1% SDS and 50% sucrose) loaded into the wells of 2% prepared agarose and fractionated in TAE buffer (40 mMTris-HCL, 20mM acetic acid and 1mM EDTA) in a submarine agarose electrophoresis unit (HE 33, from Hoefer S. F., CA and USA). The gels were stained with ethidium bromide (0.5 μg/ml in running buffer) for each agarose. The DNA marker, kilo base pair (kbp) (Fermentas, life science). After electrophoresis, agarose gel was visualized under UV light using the transilluminator FBTIV-88 (from fisher scientific Pittsburg USA). Gels were pictured using the digital camera. **(Ashok *et al.*, 2017)**.

Results

There were 25 males (50%) and 25 females (50 %) out of 50 patients. The age of the patients ranged (21-63) years with mean ± SE (50.4 ± 1.57). Baseline characteristics also are summarized with laboratory investigations before treatment: complete blood count, bilirubin, liver enzymes, INR, albumin, Alpha

Fetoprotein and HCV RNA in serum or plasma by a sensitive qualitative and quantitative molecular method. Also, the dose of treatment ranged (400-1,200) mg/kg with mean ± SE (844 ± 22.34). **Table 1** shows the demographic, clinical, biochemical and virological characteristics of patients.

Table 1: Basic characteristic and laboratory results of patients before treatment with Mean and standard error (SE).

Variables before treatment	Mean	SE
HB	12.86	0.28
WBCs	6.09	0.37
RBCs	4.62	0.07
Plt	182.76	12.05
Alb	4.08	0.08
SGOT(AST)	46.3	4.16
SGPT(ALT)	47.3	4.37
BIL	0.84	0.04
INR	1.16	0.03
Dose	844	22.34
AFP	5.45	0.59
FBS	106.2	6.25
RNA quantitative	1470126.8	245434.3
TSH	2.46	0.4
Age (years)	50.4	1.57
Height	162.86	1.25
Weight	83.5	2.79

Abbreviations: HB: Hemoglobin; WBCs: White blood Cells; RBCs: Red blood cells; Plt: Platelets; Alb: Albumin; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; BIL: Bilirubin; INR: International Normalized Ratio; AFP: Alfa fetoprotein; FBS: Fasting blood sugar; RNA: Ribonucleic acid and TSH: Thyroid stimulating hormone.

Baseline characteristics before treatment (T0), one week after treatment (T1), one month after treatment (T2) and 3 months after treatment (T3) of all patients are summarized in **Table 2**. Data of all patients in different periods were analyzed using univariate analysis.

Concerning hemoglobin, there was a significant decrease between T0 and T3 (P3=0.007**, table 2 and Figure1**). In Concerning Red blood cells, there was a significant decrease between T0 and T3 (P3=0.006*, **table2 and Figure2**). In White blood cells, there was an increase in WBCs after one week and after one month of treatment then the reduction in the count occurred after 3 months of treatment **Figure3**. Platelets count increased after a week and after one month of treatment then decreased after 3 months of treatment **Figure 4**.

Table 2: Basic characteristics and comparison of laboratory results after and before treatment. Values represent mean±SE.

|	T0	T1	T2	T3	Post hoc Tukey test
HB	12.86 ± 0.28	12.41 ± 0.24	12.13 ± 0.25	11.67 ± 0.26	P1=0.607;P2=0.19;P3=0.007*
WBCs	6.09 ± 0.37	6.41 ± 0.3	6.38 ± 0.31	5.65 ± 0.29	P1=0.890;P2=0.917;P3=0.775
RBCs	4.62 ± 0.07	4.5 ± 0.08	4.35 ± 0.09	4.24 ± 0.09	P1=0.716;P2=0.087;P3=0.006*
Plt	182.76 ± 12.05	194.26 ± 15.85	195.44 ± 16.21	187.51 ± 15.64	P1=0.949;P2=0.933;P3=0.996
Alb	4.08 ± 0.08	3.93 ± 0.11	3.96 ± 0.07	4.03 ± 0.07	P1=0.585; P2=0.712; P3=0.961
SGOT	46.3 ± 4.16	38.4 ± 3.32	26.74 ± 1.52	27.6 ± 1.4	P1=0.208; P2<0.001*; P3<0.001*
SGPT	47.3 ± 4.37	39.02 ± 3.61	25.8 ± 1.59	25.52 ± 1.66	P1=0.225; P2<0.001*; P3<0.001*
BIL	0.84 ± 0.04	0.88 ± 0.05	0.85 ± 0.05	0.76 ± 0.03	P1=0.906; P2=0.991; P3=0.639
INR	1.16 ± 0.03	1.14 ± 0.03	1.16 ± 0.03	1.12 ± 0.02	P1=0.960; P2=1.0; P3=0.737
Dose	844 ± 22.34	-	804 ± 23.90	787.76 ± 25.69	P2=0.465; P3=0.226

P1: T0 vs. T1; **P2**: T0 vs. T2; **P3**: T0 vs. T3
One-Way ANOVA test, * Significant P < 0.05
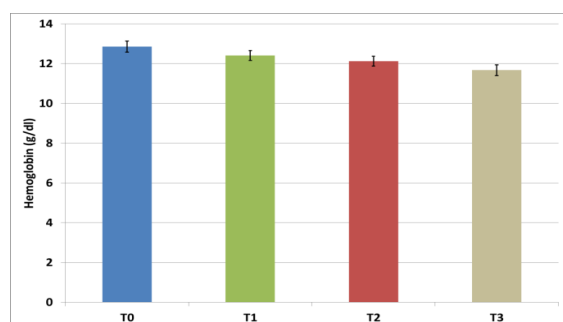
Figure 1. Hemoglobin concentration (g/dl) in HCV patients before and after treatment. Data are represented by means± SE.
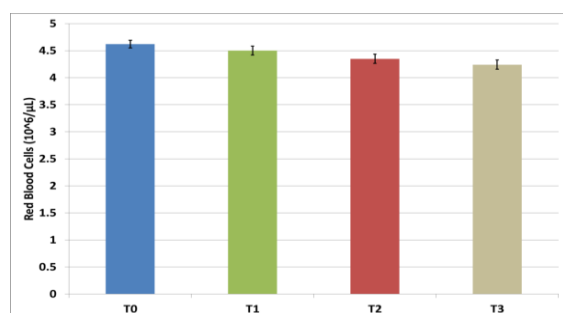
Figure 2. Red blood cells count (10^6/µl) in HCV patients through 3 months of treatment. Data are represented by means± SE.
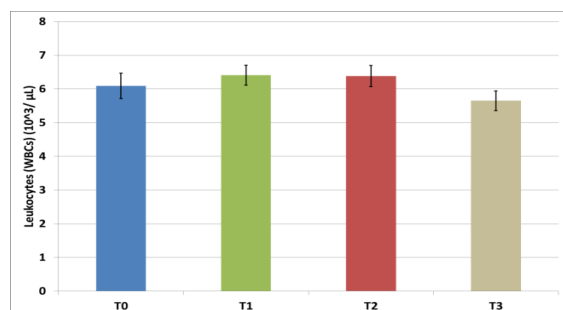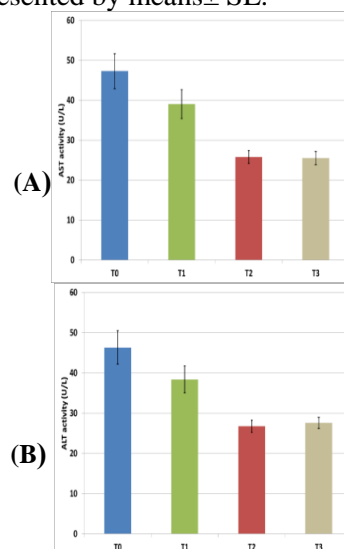

Figure 3. White blood cells count (10^3/µl) in HCV patients through 3 months of treatment. Data are represented by means± SE.
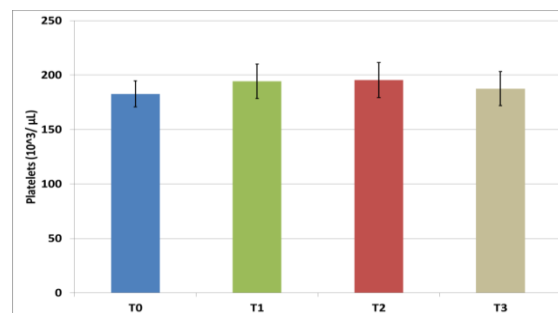

Figure 4. Platelets count (10^3/µl) in HCV patients through 3 months of treatment. Data are represented by means± SE.

In terms of liver functions, there was a significant decrease in activity of AST **(A)** and ALT **(B)** between (T0, T2) and (T0, T3) respectively (P2<0.001*, P3<0.001*) and (P2<0.001*, P3<0.001*), **Fig. 5 (A) and (B)**. Total bilirubin **Fig. 6** as well as Albumin **Fig. 7** did not show any significant differences .Data are represented by means± SE.

Figure 5. Activity of AST in U/L (A) and activity of ALT in U/L (B) in HCV patients through 3 months of treatment. Data are represented by means± SE.
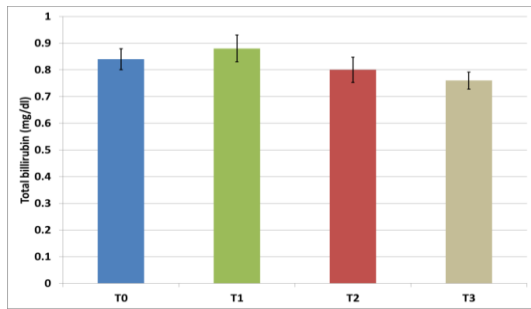
Figure 6. Concentration of total bilirubin mg/dl in HCV patients through 3 months of treatment. Data are represented by means± SE.
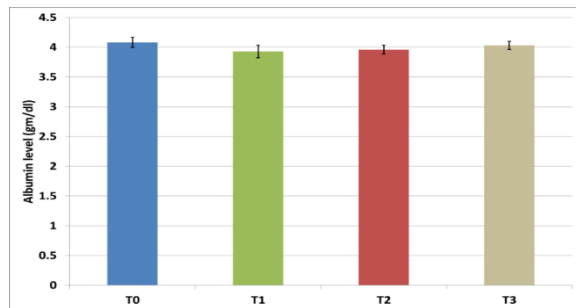
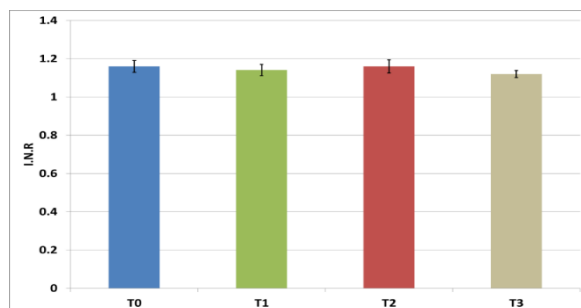**Figure 7.** Concentration of serum albumin g/dl in HCV patients through 3 months of treatment. Data are represented by means± SE.

INR did not show any significant differences in HCV patients through 3 months of treatment **Fig.8.**

Figure 8. INR values in HCV patients through 3 months of treatment. Data are represented by means± SE.
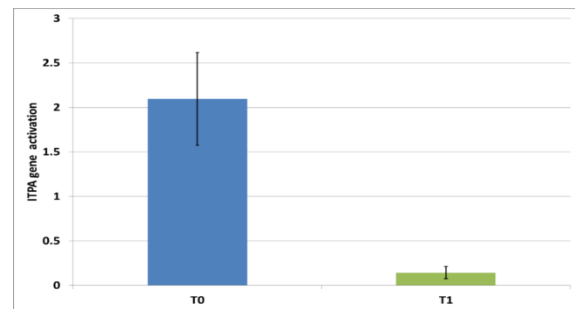
ITPA gene expression before and after treatment is shown in **table 3**, **Figure 9**. The

activation of ITPA gene decrease significantly after one month treatment as compared to before treatment (P<0.001*).

Figure 9. ITPA gene activation before and after one month of treatment. Data are represented by means± SE.

There was a reduction in ITPase activity resulting in RBCs hemolysis. The rest of variables did not show any significant through reduction of ITPA gene activity.

There was an inverse relationship between gene and dose resulted in reducing gene activity after one month of treatment. The other variables did not show any significant differences after treatment.

There were no differences between types of treatment. The table shows Mean±SE of two types of treatment and their relations with hemoglobin after and before treatment.

PCR amplification of ITPA gene

By using ITPA gene-specific primer, an amplicone with about 250 bp was amplified in PCR reaction (Figure 10). The PCR products were electrophoresis in 1.5 % agarose gel. The activity of the ITPA gene is proportional to the intensity of illumination of the bands. ITPA gene with high activity had strong illuminated bands, unlike the low activation of ITPA gene had weak illuminated bands **Fig.10**.

Table 3: Mean ± standard error and median of ITPA gene expression before and after one month of treatment.

		T0	T1		P
ITPA	**Mean ± Std. Error**	2.097 ± 0.521	0.143 ± 0.068	t=3.962	<0.001*
	Median (min – max)	0.366 (0 – 18.38)	0.0023 (0 – 2.64)	Z=5.712	<0.001*

Paired t-test; Z: Wilcoxon sign rank test * Significant P < 0.05

(Table 4) Correlations of serum ITPA gene with hematological and biochemical parameters in studied patients before treatment.

	ITPA gene before treatment	
	r	P
HB	-0.227	0.138
WBCs	-0.007	0.963
RBCs	-0.404	0.006*
Plt	-0.042	0.785
Alb	-0.041	0.791
SGOT	-0.077	0.621
SGPT	-0.192	0.212
BIL	-0.128	0.406
INR	0.356	0.018*
Dose	-0.072	0.642
AFP	0.064	0.683
FBS	-0.004	0.980
RNA quantitative	0.119	0.441
TSH	0.009	0.952
Age	0.161	0.296
Height	0.011	0.944
Weight	-0.012	0.938

Spearman's correlation co-efficient
* Significant P < 0.05

Table 5: Correlations of serum ITPA gene with hematological and biochemical parameters in studied patients after one month of treatment.

	ITPA gene After month	
	r	P
HB	-0.207	0.177
WBCs	-0.007	0.961
RBCs	-0.301	0.047*
Plt	-0.059	0.705
Alb	-0.173	0.261
SGOT	-0.025	0.871
SGPT	-0.163	0.291
BIL	0.040	0.797
INR	0.090	0.562
Dose	-0.308	0.044*

Spearman's correlation co-efficient
* Significant P < 0.05

(Table 6) Correlations of treatment with hemoglobin before, after one week, after one month and after 3 months of treatment with statistically significant decrease in hemoglobin.

	SOF/DAC/RBV (n = 30)	Qurevo/RBV (n = 20)	t	P
T0-HB	12.86 ± 1.92	12.85 ± 2.087	0.017	0.986
T1-HB	12.227 ± 1.741	12.675 ± 1.704	0.899	0.373
T2-HB	12.105 ± 1.785	12.155 ± 1.709	0.098	0.922
T3-HB	11.7 ± 2.034	11.625 ± 1.612	0.138	0.891

t: student t-test,* Significant P < 0.05

Figure 10. Reverse Trascriptase PCR (RT-PCR) amplification of the ITPA gene; Lane M: 100 bp DNA ladder, Lane 1 indicates to control, lanes (2, 3, 8, 10, 12) are weak illuminated bands that indicate to the low activity of ITPA gene after one month of treatment and lanes (4, 5, 6, 11, 13) are strong illuminated bands that indicate to the high activity of ITPA gene before treatment in HCV patients.

Discussion

The present study indicated to the hematological abnormalities in patients with hepatitis C virus treated with ribavirin therapy and their relationship with ITPA gene expression. One of the most important reasons for decreased adherence to treatment is the development of side effects such as multiple hematological abnormalities including neutropenia, anemia, and thrombocytopenia (**McHutchison *et al*., 2007 and Reau *et al*., 2008**). It demonstrates that genotype 1 is an important factor for development of hematological abnormalities in patients with hepatitis C receiving treatment. The antiviral treatment is the standard of care for treatment of chronic hepatitis C (**Kowdley *et al*., 2005**). Genotype 1 has been shown in previous studies to be a factor in influencing viral response rates to treatment. This study provides evidence to clinicians that patients with genotype 1 infection may need a closer follow-up during treatment for hepatitis C for development of hematological abnormalities. Other factors associated with hematological abnormalities include a low baseline platelet count as well as a low baseline hemoglobin count. This large cohort study in a real world setting found that genetic polymorphisms in the ITPA gene, resulting in ITPase deficiency, were associated with less Hb decline during RBV therapy among Caucasian patients with chronic HCV infection. Patients with normal ITPase activity were at higher risk to undergo dose reductions of RBV and receive blood transfusions. Patients with ITPase deficiency experienced lower declines in Hb concentration than patients with normal ITPase activity during the first four weeks of antiviral treatment. As expected,

besides the presence of normal ITPase activity, RBV dose was also associated with a significant decline in Hb at week 4. The results of the present study confirm observations described in previous studies (**Thompson** *et al*.**, 2011; Holmes** *et al*.**, 2014 and Rembeck** *et al*.**, 2014)**. In contrast, another study among 546 patients showed no association between ITPase deficiency and RBV levels (p = 0.11) (**Holmes** *et al*.**, 2014)**. The studies among patients with HCV genotype 2 and 3 found no association between the ITPase activity and the need for RBV dose reductions (**Thompson** *et al*.**, 2011 and Rembeck** *et al*.**, 2014)**. Combination therapy with ribavirin for the treatment of patients with chronic HCV is widely used as a standard regimen, because several studies attest to its prominent efficacy and the acceptable tolerability of ribavirin. (**Reichard** *et al*.**, 1997; McHutchinson** *et al*.**, 1998 and Poynard** *et al*.**, 1998)**. However, the most notable side effect of ribavirin is reversible hemolytic anemia, (**Bodenheimer** *et al*.**, 1997; Davis** *et al*.**, 1997; Reichard** *et al*.**, 1997; McHutchinson** *et al*.**, 1998; Poynard** *et al*.**, 1998; Tappero** *et al*.**, 1998; Maddrey, 1999; McHutchison** *et al*.**, 1999 and Van** *et al*.**, 2001)** which is observed as a decrease in Hb levels (2–4) weeks after the beginning of the combination therapy (**Maddrey, 1999)** and this agreed with our results. The ribavirin-induced anemia can increase fatigue, has a demonstrable effect on quality of life, and is a frequent indication for dose reduction of ribavirin. Therefore, careful monitoring should be done during the treatment, especially during the first 4 weeks. RBV induces dose-related hemolytic anemia that impairs the patients' quality of life and frequently requires a dosage reduction, which may be serious in difficult patients, such as the elderly and cirrhotic patients. This adverse reaction has been reported in more than 30% (**Stickel** *et al*.**, 2012)** of the cases treated with combination therapy, more frequent during the administration of triple combination therapy with RBV and telaprevir or boceprevir (**Piccinino** *et al*.**, 2007)** and less frequent in DAA/RBV regimens (**Coppola** *et al*.**, 2014 and Cheung** *et al*.**, 2016)**. Erythrocyte hemolysis is considered the main cause of RBV-induced anemia (**Younossi** *et al*.**, 2016)**. By reducing adenosine triphosphate (ATP) levels in human erythrocytes, RBV induces a guanosine triphosphate (GTP) depletion followed by the inhibition of the ATP-dependent oxidative

metabolism, membrane damage and premature hemolysis of erythrocytes (**De Franceschi** *et al*.**, 2000)**. In patients with reduced ITPase activity, however, inosine triphosphate (ITP) accumulates in erythrocytes (**Sumi** *et al*.**, 2002 and Arenas** *et al*.**, 2007)**, replacing the GTP activity and producing adenosine monophosphate (AMP) (**Hitomi** *et al*.**, 2011)**, thus avoiding the inhibition of the ATP-dependent oxidative metabolism and erythrocyte hemolysis. Therefore, RBV-induced anemia seems primarily to be due to the reduced levels of ATP in erythrocytes consequent to the effect of the drug on GTP (**Jimmerso** *et al*.**, 2016)**, and resistance against RBV-related anemia is due to a reduced ITPA activity (**Thompson** *et al*.**, 2011)**.

Conclusion

The patients with deficiency in ITPA gene expression showed a decline in hemoglobin during the first month of treatment. ITPA polymorphisms increase the likelihood of developing hemolytic anemia for HCV-infected patients on RBV-based therapy, suggesting the utility of screening for ITPA polymorphisms to avoid hematological toxicity and increase adherence to RBV-based therapy.

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الملخص العربي

عنوان البحث: ارتباط نشاط جين الإتبا بالتشوهات الدموية المستحثة بالعلاج الثنائي في المرضي المصابين بالالتهاب الكبدي الفيروسي- سي

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الملخص العربي

يُعد فيروس التهاب الكبد الوبائي (سي) أحد الأسباب الرئيسية لتليف الكبد وسرطان الخلايا الكبدية. يمكن تحقيق الاستجابة الفيروسية المستمرة ، التي تُعرّف بأنها سلبية للحمض النووي الريبوزي لفيروس التهاب الكبد الوبائي (سي) لمدة ٢٤ أسبوعًا بعد التوقف عن العلاج ، من خلال نظام العلاج الحالي باستخدام الريبافيرين في العلاج المركب. يعتبر انحلال الدم أحد الآثار الجانبية الشائعة للريبافيرين وهو السبب الرئيسي لخفض الجرعة. تظهر التشوهات الدموية بشكل شائع في المرضى الذين يعانون من التهاب الكبد الوبائي المزمن الذين تم معالجتهم بالعلاج المركب الذي يحتوي على الريبافيرين. وتشير الدراسات الحديثة إلى أن العلاج المركب الذي يحتوي علي الريبافيرين فعال لعدوى فيروس التهاب الكبد الوبائي سي (HCV). يؤدي فقر الدم الناجم عن الريبافيرين (RBV) في المقام الأول إلى تقليل مستويات الأدينوزين ثلاثي الفوسفات (ATP) في كريات الدم الحمراء ، مما يؤثر على التمثيل الغذائي التأكسدي المعتمد على ATP. ترتبط الاختلافات الجينية في جين إينوزين ثلاثي الفوسفاتيز (ITPA) ، الذي يشفر إينوزين ثلاثي الفوسفات بيروفوسفو هيدرولاز (ITPase) ، بالحماية من فقر الدم الانحلالي أثناء العلاج بالريبافيرين. تؤثر متغيرات جين الايتبا (ITPA) هذه على وظائف إنزيم الايتبا (ITPase)) ، مما يتسبب في انخفاض نشاطها ، مما يؤدي إلى تراكم إينوزين ثلاثي الفوسفات (ITP) في كريات الدم الحمراء ومنع الإجهاد التأكسدي. يتم متابعة جميع المرضى المعالجين في الأسبوع الأول والرابع من العلاج وبعد ذلك في الشهر الثالث بعد توقفه لتقييم الاستجابة الفيروسية. في هذه الدراسة تم استخدام العلاج المضاد للفيروسات خاصة (كيوريفو و الريبافيرين) و (سوفوسبوفير و داكلاتسفير و الريبافيرين) عن طريق الفم ولا يوجد فرق كبير بين مجموعات العلاج السابقة. هدفت هذه الدراسة إلى توضيح العلاقة بين نشاط جين الإتبا والآثار الجانبية الدموية التي تحدث أثناء استخدام العلاج الثنائي بالريبافيرين في المرضى الذين يعانون من تليف الكبد المرتبط بفيروس التهاب الكبد الوبائي (سي).