Received: 3 February 2017 | Accepted: 11 April 2017
DOI: 10.1002/jmv.24844

RESEARCH ARTICLE

WILEY JOURNAL OF MEDICAL VIROLOGY

ITPA gene polymorphism (*94C>A*) effects on ribavirin-induced anemia during therapy in Egyptian patients with chronic hepatitis C

Maissa El Raziky[1] | Naglaa A. Zayed[1] | Amin Abdel Baki[2] | Shimaa A. Mansour[2] | Rasha M. H. Shahin[3]

[1] Department of Endemic Medicine and Hepatology, Kasr Al Ainy Hospital, School of Medicine, Cairo University, Cairo, Egypt

[2] Department of Tropical Medicine, National Hepatology and Tropical Medicine Research Institute (NHTMRI), Cairo, Egypt

[3] Department of Clinical Pathology, Kasr Al Ainy, School of Medicine, Cairo University, Cairo, Egypt

Correspondence
Rasha M. H. Shahin, Assistant Professor of Immunology, Department of Clinical Pathology, School of Medicine, Cairo University, 11c, Street 199, Apt 9, Degla, Maadi, Cairo, Egypt.
Email: rasha.shaheen@kasralainy.edu.eg

Funding information
Grant From Bilharzial Liver Unit, Tropical Department, Cairo University

Inosine triphosphatase (ITPA) gene variants can protect against ribavirin (RBV)-induced anemia in patients treated for chronic hepatitis C. The aim of this study was to determine the relationship between genetic variants of ITPA polymorphism, anemia, RBV dose reduction, and treatment response in hepatitis C virus (HCV)-infected patients. This study was conducted on 97 Egyptian chronic HCV patients who were scheduled for pegylated-interferon (PEG-INF) /RBV therapy. ITPA genotypes *rs1127354* were determined by Real Time PCR melting curve analysis. Effects of ITPA polymorphism on hemoglobin (Hb) levels, RBV dose reduction and treatment response were analyzed. The homozygous wild genotype (*CC*) was associated with Hb reduction at week 4 ($P = 0.004$). The minor allele protected against Hb reduction. No association with sustained virological response was observed ($P = 0.492$). Female gender; lower baseline Hb and higher baseline WBC were associated with week 4 anemia ($P = 0.04$; $P = 0.023$; 0.033, respectively). The ITPA gene polymorphism *rs1127354* heterozygous genotype (*CA*) may influence Hb levels and protect against hemolytic anemia during RBV-containing regimens for HCV. However, such findings were not significantly related to treatment outcomes. Patients with wild ITPA genotype (*CC*) experienced a more Hb drop and RBV dose reductions more frequently.

KEYWORDS
anemia, chronic hepatitis C, ITPA polymorphism, ribavirin

1 | INTRODUCTION

Worldwide, 130-170 million people are infected with hepatitis C virus (HCV).[1] Globally, it was found that HCV is responsible for 27% and 25% of cirrhosis and hepatocellular carcinoma (HCC) patients, respectively.[2] The highest prevalence rate of HCV infection in the world is present in Egypt, evaluated nationally at 14.7%.[3]

For many decades, the standard of care treatment was the combination of pegylated interferon (PEG-IFN) and ribavirin (RBV) until the introduction of directly acting anti-viral therapy (DAA). With the change in paradigm in HCV treatment, RBV is still an important component of IFN-containing regimens, and is also included in many IFN-free regimens currently in development.[4–7] Even in the era of DAAs, RBV is still favored in different combination regimens as another option to prolonging duration of therapy.[8] RBV (a guanosine analog) is an antiviral drug acting through the interruption of the metabolism of viral RNA .[9]

Cytopenias, including RBV-induced hemolytic anemia, are one of the crucial side effects that have been frequently associated with

PEG-IFN and RBV. Moreover, RBV-induced hemolytic anemia has been detected in IFN-free, RBV-containing clinical trials.[6,7,10,11] RBV-induced hemolytic anemia often results in reduction or discontinuation of RBV dose which may ultimately compromise treatment efficacy. Lowering the RBV dosage may decrease the chance of sustained virologic response (SVR) and increase the rate of relapse.[12] RBV-induced anemia is likely to be dose-dependent as most patients show improved hemoglobin (Hb) levels with the reduction of RBV dose and it is reversible after the end of treatment.[13] The myelosuppressive action of PEG-IFN may worsen RBV-hemolytic anemia.[6,7,10,11]

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[14] and eventual protection against RBV-induced hemolytic anemia.[15] 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.[16]

ITPA hydrolyzes inosine triphosphate (ITP) and deoxyinosine 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.[14]

In this study, the impacts of ITPA variant *rs1127354* on RBV-induced anemia, RBV dose reduction or treatment discontinuation were investigated in Egyptian HCV patients treated with PEG-IFN plus RBV.

2 | SUBJECTS AND METHODS

2.1 | Subjects

This study was performed on a sample of 97 Egyptian HCV infected patients; 71 males (73.2%) and 26 females (26.8%), with mean ± SD age 45.17 ± 9.73 years; recruited from Hepatology clinic, National Hepatology and Tropical Medicine Research Institute (NHTMRI), Ministry of Health and Population (MOHP), Egypt. These patients were tested for eligibility of PEG-INF/weight adjusted dose of RBV for 48 weeks, according to the program of The National Committee for Control and Prevention of Viral Hepatitis, Ministry of Health and Population (MOHP), Egypt.

Patients were diagnosed for positivity of HCV antibody using a 2nd generation enzyme-linked immunosorbent assay (ELISA), positivity of HCV RNA by polymerase chain reaction (PCR) and liver biopsy. HCV genotyping was determined using reverse hybridization line probe assay (INNO LiPA HCV II kit, Innogenetics, Belgium).

The patients were evaluated for antiviral therapy, according to national guidelines for HCV, including the following inclusion and exclusion criteria.

- *Inclusion criteria:* Treatment-naïve chronic HCV patients aged 18-60 years old, BMI ≤ 35, serologic, virologic, and histologic diagnosis of chronic HCV.
- *Exclusion criteria*: Decompensated hepatic disease, hepatitis B surface antigenemia (HBsAg) or infection with human immunodeficiency virus (HIV), serum creatinine above upper limit of normal, hemoglobin <13 g/dL for men and <12 g/dL for women, white blood cell count of <4000/mm^3 or neutrophil count of <2000/mm^3, poorly controlled diabetes mellitus or hypertension or under care of a psychiatrist.

The concept of the study was clearly explained to all participants who then provided informed consent. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by both the National Committee for Prevention and Control of Viral Hepatitis and national Hepatology and tropical medicine research institute (NHTMRI).

All 97 patients were treated with PEG-INF-alfa once weekly and initial dose of 800-1400 mg RBV daily according to the patient's weight. Dose reduction of RBV by 50% and 35% was carried in eight patients reaching 600 mg/day and 27 patients reaching 800 mg/day, respectively.

Treatment response (achievement of SVR) was defined by undetectable serum HCV RNA by real time PCR (<50 IU/mL) 24 weeks after the completion of therapy.[17]

All patients were subjected to initial baseline evaluation in the form of full history taking, proper physical examination, laboratory investigations, abdominal ultrasound, histopathological examination of liver biopsy according to METAVIR scoring system[18] and determination of ITPA *94C>A rs 1127354* gene polymorphism by real-time PCR.[19]

All participants were scheduled to weekly follow up in the first month of treatment then monthly for 48 weeks. Follow up included clinical, laboratory assessment (CBC, serum bilirubin, liver enzyme, serum creatinine), treatment dose modifications or discontinuation, virological response at week 12, 24, 48, and 72. Clinically significant decline in Hb was defined as a decrease of at least 3.0 g/dL or an absolute value lower than 10 g/dL.[20] These thresholds were also used in other studies on ITPase deficiency.[21–23]

3 | METHODS

3.1 | Genotyping of ITPA *94C>Ars 1127354* polymorphism by real-time PCR

Two milliliters of blood were put into vaccutainers containing ethylene diamine tetra-acetic acid (EDTA) for DNA extraction and analysis of *ITPA94C>A* polymorphism using Real time PCR-assay.

Total genomic DNA of patients was extracted using Qiagen extraction kit (catalog number 51104, USA).

Forward (*5′-CTT TAG GAG ATG GGC AGC AG-3′*) and reverse (*5′-CAC AGA AAG TCA GGT CAC AGG-3′*) primers were utilized for amplification of selected region in *ITPA* gene. Monitoring the accumulation of specific PCR product is by Hybridization probes. One probe was labeled with fluorescein (FLU) at 3′ end, while 5′ end of an adjacent anchor probe was labeled with Cy5.5. 3′-phosphorylation prevented anchor probe elongation by *Taq* polymerase. All oligonucleotides were manufactured by Roche (Germany). Fluorescence resonance energy transfer (FRET) occurs when both probes hybridize closely and is detected by the Light Cycler.

The classification of ITPA *94C>A* gene as homozygous *wild* (*CC*), polymorphic homozygous (*AA*), or heterozygous (*CA*) was performed by a melting curve analysis.

Slowly increasing the temperature of reaction causes dissociation of the probe from its target accompanied by a loss of fluorescence resulting in a temperature/fluorescence curve from which the melting point of the probe can be known.[19]

The 3′-FLU-tagged probe: *5′AGT TTC CAT GCA CTT TGG 3′* and 5′-CY5.5-tagged anchor probe: *5′GGC ACA GAA AAT TGA CCG TAT GTC TC 3′* were utilized for The *ITPA* 94C wild type. They were made to get the maximal difference in the melting temperature between the genotypes.

One low temperature peak (55°C) indicates the homozygous wild genotype (*CC*). Double peaks indicate a heterozygous genotype (*CA*). A single high temperature melting peak (62°C) indicates homozygous minor genotype (*AA*).

PCR reactions were carried out in Light Cycler glass capillaries. The20 μL reaction mixture consisted of 1-5 μL (50 ng) of DNA solution, 1.6 μL $MgCl_2$, 14.4-10.4 μL H_2O, 1.0 μL reagent mix (one reagent vial contains all primers and probes to run 97 Light Cycler reactions), 2.0 μL Fast Start DNA Master and a Final $MgCL_2$ conc 3.0 mM (Roche Diagnostics).

3.2 | HCV genotyping

The line probe assay was utilized to evaluate HCV genotypes, which depends on genotype-specific oligonucleotides that are immobilized on a nitrocellulose strip. The 5′ untranslated (UTR) core region of HCV was amplified using reverse transcriptase (RT-PCR), and the oligonucleotides were probed with a biotin-tagged 5′ UTR amplicon. After washing, streptavidin labeled with alkaline phosphatase was utilized to detect the hybridized products, and BCIP/NBT chromogen was used as a substrate as indicated by the manufacturer's instructions. The probe reactivity patterns were interpreted using the chart provided by the manufacturer.[24]

3.3 | Statistical analysis

The data were evaluated in statistical program SPSS version 16.0. Qualitative data included descriptive statistics (frequency and percentage of categorical parameters were presented as No, %), Fisher's exact and chi-square which were applied with 95% confidence interval. Continuous variables were expressed as Mean ± Standard Deviation and Student's test (2 tailed) which was used to compare the means. *P* value <0.05 was considered statistically significant for all comparisons.

4 | RESULTS

Clinically significant RBV-induced anemia; a drop of 3g/dL of Hb at week 4 from the initial level in patients on a RBV weight-adjusted dose; occurred in 46 (47.4%) of treated HCV patients.

At week 4 of treatment, female gender; lower baseline Hb and higher baseline WBC were more common in patients who developed RBV induced anemia. Factors associated with week 4 anemia are shown in Table 1.

Reduction of RBV doses occurred during the course of treatment as a result of development of anemia. Patients who developed anemia received lower doses of RBV and the difference was statistically significant as shown in Table 2.

4.1 | ITPA *(94C>A)* genotypes distribution

We genotyped ITPA *rs1127354* in 97 HCV-infected patients. Eighty-eight (91%) patients possessed ITPA *rs1127354* homozygous wild genotype (*CC*) and nine (9%) patients possessed ITPA *rs1127354* heterozygous genotype (*CA*).

ITPA *rs1127354* wild type was significantly associated with more RBV-induced anemia at week 4 than ITPA *rs1127354* minor type in Egyptian patients during PEG-INF plus RBV treatment (Table 3).

Sustained virilogical response (SVR) was achieved in 51 (52.6%) patients. Treatment response was compared among patients according to ITPA genotypes; ITPA *rs1127354* was not significantly related to SVR (Table 4). We compared RBV doses among patients according to ITPA genotypes. ITPA *rs1127354* was not related to RBV doses (Table 4).

HCV genotype 4 was only detected in our patients.

5 | DISCUSSION

It is worthwhile that we can predict RBV induced anemia among chronic HCV and various prognostic factors have been proposed.

Several related studies have been conducted, expressing the association between genetic variants of the ITPA gene and RBV induced anemia. Chronic HCV patients with lower ITPA enzyme activity have a less likelihood of facing hemolytic anemia during RBV-treatment regimens.[20,25,26]

In our study, a functional single nucleotide polymorphism (SNP) in ITPA gene *rs1127354* was associated with RBV induced anemia among 97 Egyptian patients. Of these 97 patients, 88 (91%) had the RBV-sensitive *CC* genotype, and 9 (9%) the RBV-resistant *CA* genotype.

Our results coincide with those of European,[25,27–29] Asian,[26,30,31] and Brazilian studies.[13] On the other hand, these frequencies were distinct from those reported by Fellay et al[15] who showed that 47.6% of Americans had the *CC* genotype.

TABLE 1 Factors associated with week 4 clinically significant anemia in HCV patients receiving PEF-IFN and weight-based RBV therapy

	Anemia N (%)		
Variable (No.)	Yes (*n* = 46)	No (*n* = 51)	*P* value
Sex [No (%)]			
Male (71)	29 (40.8)	42 (59.2)	0.040*
Female (26)	17 (65.4)	9 (34.6)	
Fibrosis [No (%)]			
≤F2 (63)	31 (49.2)	32 (50.8)	0.67
>F2 (34)	15 (44.1)	19 (55.9)	
Age (yrs) (Mean ± SD)	46.33 ± 10.49	44.14 ± 8.98	0.271
Baseline WBCs (Mean ± SD)	7.01 ± 1.89	6.17 ± 1.91	0.033*
Baseline Hb (g/dl) (Mean ± SD)	13.7 ± 1.44	14.5 ± 1.56	0.023*
Baseline PLT (Mean ± SD)	209.65 ± 59.06	206.31 ± 50.38	0.765
Baseline ALT (U/L) (Mean ± SD)	71.90 ± 36.25	76.45 ± 45.59	0.590
Baseline AST (U/L) (Mean ± SD)	58.34 ± 27.60	59.26 ± 29.52	0.875
Baseline ALP (U/L) (Mean ± SD)	115.31 ± 43.21	101.91 ± 39.14	0.112
Baseline Total Bilirubin (mg/dl) (Mean ± SD)	0.75 ± 0.29	0.81 ± 0.34	0.397
Baseline Albumin (g/dl) (Mean ± SD)	4.09 ± 0.39	4.19 ± 0.39	0.238
Baseline INR (Mean ± SD)	1.23 ± 1.18	1.05 ± 0.10	0.285
Baseline TSH (Mean ± SD)	2.05 ± 2.18	1.67 ± 0.94	0.262
Baseline HCVRNA (10^5) (IU/mL) (Mean ± SD)	4.250 ± 7.4	90.96 ± 2.59	0.227

*$P < 0.05$ is considered statistically significant.

Interestingly, the distribution of different ITPA genotypes in our cohort group was compatible with two other Egyptian studies that reported that 93% of the studied populations were *CC* genotype.[32,33]

Many factors, including ITPA gene SNP *rs 1127354*; female gender; old age; amount of the drugs; baseline platelets and Hb levels, were reported to be associated with evolvement of anemia during antiviral therapy.[34]

Scherzer et al[35] in their study detected gender differences in the development of RBV-hemolytic anemia at week 4 during treatment of Austrian patients with PEG-IFN/RBV.

In our study, female gender; lower baseline Hb; higher baseline WBC, and *rs1127354*, were independently associated with RBV induced anemia. We found that *rs1127354* might be a useful prognostic marker of RBV induced anemia. ITPA *rs1127354* wild type led to significantly greater RBV-induced anemia at week 4 than ITPA *rs1127354* minor type. That is to say that the minor allele was associated with protection against week 4 anemia.

TABLE 2 RBV dose reduction associated with anemia among HCV patients on combined Peg-IFN/RBV therapy

	Anemia		
	No (51)	Yes (46)	
Variable (unit)	Mean ± SD	Mean ± SD	*P* value
RBV dose	1082.35 ± 126.20	1018.86 ± 144.48	0.023*

*$P < 0.05$ is considered statistically significant.

The problem with RBV induced hemolytic anemia that it frequently leads to RBV dose reduction. This was the case in our study; patients who developed anemia received lower doses of RBV.

In our study, we compared the treatment response among patients according to ITPA genotypes. Although patients having minor allele (with reduced ITPase activity) were protected from week 4 RBV induced anemia; were in less need for RBV dose reductions, these were not reflected on SVR rates.

We did not detect any association between ITPA genotypes and the attainment of SVR. This finding is in agreement with the studies of Thompson et al,[20] Miyamura et al,[36] Kim et al,[26] and Delvaux et al[13] Moreover, our results are consistent with the largest study conducted

TABLE 3 Serial analysis of Hb% during 12 weeks of treatment according to ITPA genotypes

	ITPA rs1127354		
	CA (9)	CC (88)	
	Mean ± SD	Mean ± SD	*P* value
Hb (w0)	14 ± 1.81	14.2 ± 1.46	0.713
Hb (w1)	13.8 ± 1.73	13.6 ± 1.45	0.775
Hb (w2)	13.2 ± 1.82	12.5 ± 1.33	0.194
Hb (w4)	13 ± 1.69	11.5 ± 1.42	0.004*
Hb (w8)	12 ± 1.85	11 ± 1.36	0.059
Hb (w12)	11.8 ± 1.60	10.9 ± 1.30	0.052

*$P < 0.05$ is considered statistically significant.

TABLE 4 Treatment outcome and ribavirin doses in relation to ITPA genotypes

	ITPA *rs1127354*			
Response to treatment	**CA (9 patients)**	**CC (88 patients)**	**Total**	***P* value**
SVR	6 (11.8%)	45 (88.2%)	51 (100.0%)	0.492
Non-SVR	3 (6.5%)	43 (93.5%)	46 (100.0%)	
RBV dose	1074 ± 161.39	1050 ± 136.46		0.621

*$P < 0.05$ is considered statistically significant.

among patients with HCV genotype 1.[21] The impact of ITPase activity on virological response was inconclusive in previous studies. This could be related to the diversity of treatment regimens and HCV genotypes. However, treatment efficacy could be compromised by the assumption of higher frequency of dose reductions among patients with normal ITPase activity.

It was curious that no benefit regarding treatment outcome was observed despite this protective effect against anemia, and less need for RBV dose reduction. This could be explained by the fact that we were most likely underpowered to observe an effect on SVR as it is the minor allele that is protective and only minority of patients are protected against anemia.

The complicated relationship between anemia, *ITPA* variants, and SVR may include opposing mechanisms that need further investigations.

Patients with *CC* genotype (normal ITPase activity) might require watchful monitoring of Hb levels during therapy. This group may be supported with reduction of RBV dose and/or growth factor to minimize early discontinuation of RBV and provide safety.[37] In addition, the small group of patients with the minor allele could be checked less often as they are protected from anemia. Also, they may be subjected to more aggressive RBV dose escalation strategies, as higher-doses RBV have been linked to higher rates of SVR.[38] Prospective studies are required to assess the cost-effectiveness and usefulness of these approaches.

Our study has certain limitations. ITPA expression and ITP levels in erythrocytes were not measured.

There are seven genotypes of HCV, with at least 15% difference in coding region sequences. Treatment with PEG-IFN/RBV is individualized by genotype. Treatment regimen of HCV genotype 4 with PEG-IFN-alfa/RBV requires 48-weeks and accomplishes SVR rates of 43-70%. Patients with HCV genotype 1 require a 48 week treatment regimen and a standard dose of RBV; while those with HCV genotype 2 or 3 appear to be adequately treated with a low dose of RBV for 24 weeks.[39]

Although our patients received dual IFN/RBV therapy, which was the standard of care therapy few years ago, still RBV has a potential major role in most of INF-free, DAA therapy. Thus the assessment of any factors associated with RBV induced anemia was essential.

The effect of ITPA polymorphism on IFN-free, DAA-regimens containing RBV; sofosbuvir plus RBV[40] and *NS3/4A* protease inhibitor faldaprevir, the non-nucleoside polymerase inhibitor deleobuvir, and RBV[41] has been recently investigated. ITPA polymorphism affects Hb levels and incidence of RBV dose reduction during sofosbuvir plus RBV therapy.[40] Moreover, ITPA *rs1127354 CC* and *rs6051702 AA* genotypes may predict RBV-induced anemia during treatment with IFN-free, RBV-containing regimens. With this IFN-free regimen, SVR was associated with RBV levels, but not with ITPA genotypes or anemia.[41]

It is worth mentioning that treatment of chronic HCV is based on guidelines from Infectious Diseases Society of America (IDSA) and American Associations for the Study of Liver Diseases (AASLD), in collaboration with International Antiviral Society-USA (IAS-USA). These guidelines are constantly updated.[42]

6 | CONCLUSION

In our study, although patients with the minor allele of ITPA polymorphism *rs1127354 CA*, had less anemia, which supports the hypothesis that ITPA variants are protected against RBV induced anemia, less need for dose reduction, that benefit did not translate to clinically relevant outcomes such as SVR.

Patients who are more likely to tolerate higher doses of RBV or who are at greater risk of more severe anemia could be identified, prior to initiating therapy with DAA-containing RBV regimens, by screening for ITPA polymorphisms. Future recognition of other SNPs associated with anemia or with other adverse effects will help clinicians to tailor treatment to maximize response and minimize adverse events.

ACKNOWLEDGMENT

The study received a grant from Bilharzial liver unit, Tropical department, Cairo University. This manuscript has not been published elsewhere and it has not been submitted simultaneously for publication elsewhere.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

REFERENCES

1. Davis GL, Alter MJ, El-Serag H, Poynard T, Jennings LW. Aging of hepatitis C virus (HCV)-infected persons in the United States: a multiple cohort model of HCV prevalence and disease progression. *Gastroenterology*. 2010;138:513–521.

2. Massard J, Ratziu V, Thabut D, et al. Natural history and predictors of disease severity in chronic hepatitis C. *J Hepatol*. 2006;44:S19–S24.
3. Shaker MK, Abdella HM, Khalifa MO, El Dorry AK. Epidemiological characteristics of hepatocellular carcinoma in Egypt: a retrospective analysis of1313 cases. *Liver Int*. 2013;33:1601–1606.
4. Jacobson IM, McHutchison JG, Dusheiko G, et al. Telaprevir for previously untreated chronic hepatitis C infection. *N Engl J Med*. 2011;364:2405–2416.
5. Poordad F, McCone J, Jr, Bacon BR, et al. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med*. 2011;364: 1195–1206.
6. Gane EJ, Stedman CA, Hyland RH, et al. Nucleotide polymerase inhibitor sofosbuvir plus ribavirin for hepatitis C. *N Engl J Med*. 2013; 368:34–44.
7. Poordad F, Hézode C, Trinh R, et al. ABT-450/r-ombitasvir and dasabuvir with ribavirin for hepatitis C with cirrhosis. *N Engl J Med*. 2014;370:1973–1982.
8. European Association for the Study of the Liver (EASL) recommendations on treatment of hepatitis C 2016. *J Hepatol*. 2016. Available online at: https://doi.org/10.1016/j.jhep.2016.09.001.
9. Furusyo N, Katoh M, Tanabe Y, et al. Interferon alpha plus ribavirin combination treatment of Japanese chronic hepatitis C patients with HCV genotype 2: a project of the Kyushu University Liver Disease Study Group. *World J Gastroenterol*. 2006;12:784–790.
10. Zeuzem S, Asselah T, Angus P, et al. Efficacy of the protease inhibitor BI 201335, polymerase inhibitor BI 207127, and ribavirin in patients with chronic HCV infection. *Gastroenterology*. 2011;141: 2047–2055.
11. Schinazi R, Halfon P, Marcellin P, Asselah T. HCV direct-acting antiviral agents: the best interferon-free combinations. *Liver Int*. 2014;34:69–78.
12. Russmann S, Grattagliano I, Portincasa P, Palmieri VO, Palasciano G. Ribavirin-induced anemia: mechanisms, risk factors and related targets for future research. *Curr Med Chem*. 2006;13:3351–3357.
13. Delvaux N, da Costa VD, da Costa MM, et al. Inosine triphosphatase allele frequency and association with ribavirin-induced anaemia in Brazilian patients receiving antiviral therapy for chronic hepatitis C. *Mem Inst Oswaldo Cruz*. 2015;110:636–643.
14. Hitomi Y, Cirulli ET, Fellay J, et al. Inosine triphosphate protects against ribavirin-induced adenosine triphosphate loss by adenylosuccinate synthase function. *Gastroenterology*. 2011;140: 1314–1321.
15. Fellay J, Thompson AJ, Ge D, et al. ITPA gene variants protect against anaemia in patients treated for chronic hepatitis C. *Nature*. 2010;464:405–408.
16. Sumi S, Marinaki AM, Arenas M, et al. Genetic basis of inosine triphosphate pyrophosphohydrolase deficiency. *Hum Genet*. 2002;111:360–367.
17. Fried MW, Shiffman ML, Reddy KR, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med*. 2002;347:975–982.
18. Stanciu A, Floarea-Strat A. Knodell and Metavir scoring systems-advantages and limits. *Rev Med Chir Soc Med Nat Iasi*. 2002;106: 753–756.
19. Heller T, Oellerich M, Armstrong VW, von Ahsen N. Rapid detection of *ITPA* 94C>A and IVS2+21A>C gene mutations by real-time fluorescence PCR and in vitro demonstration of effect of *ITPA* IVS2 +21A>C polymorphism on splicing efficiency. *Clin Chem*. 2004;50: 2182–2184.
20. Thompson AJ, Fellay J, Patel K, et al. Variants in the ITPA gene protect against ribavirin-induced hemolytic anemia and decrease the need for ribavirin dose reduction. *Gastroenterology*. 2010;139: 1181–1189.
21. Holmes JA, Roberts SK, Ali RJ, et al. ITPA genotype protects against anemia during peginterferon and ribavirin therapy but does not influence virological response. *Hepatology*. 2014;59: 2152–2160.
22. Thompson AJ, Santoro R, Piazzolla V, et al. Inosine triphosphatase genetic variants are protective against anemia during antiviral therapy for HCV2/3 but do not decrease dose reductions of RBV or increase SVR. *Hepatology*. 2011;53:389–395.
23. Clark PJ, Aghemo A, Degasperi E, et al. Inosine triphosphatase deficiency helps predict anaemia, anaemia management and response in chronic hepatitis C therapy. *J Viral Hepat*. 2013;20:858–866.
24. Bouchardeau F, Cantaloube J, Chevaliez S, Portal CH, Razer A, Lefrère JJ. Improvement of hepatitis C virus (HCV) genotype determination with the new version of the INNO-LiPA HCV assay. *J Clin Microbiol*. 2007;45:1140–1145.
25. Naggie S, Rallon NI, Benito JM, et al. Variants in the ITPA gene protect against ribavirin-induced hemolytic anemia in HIV/HCV-coinfected patients with all HCV genotypes. *J Infect Dis*. 2012;205:376–383.
26. Kim JS, Ahn S-M, Jung YK, et al. The impact of inosine triphosphatase variants on hemoglobin level and sustained virologic response of chronic hepatitis C in korean. *J Korean Med Sci*. 2013;28:1213–1219.
27. Domingo P, Guardiola JM, Salazar J, et al. Association of ITPA gene polymorphisms and the risk of ribavirin-induced anemia in HIV/hepatitis C virus (HCV)-coinfected patients receiving HCV combination therapy. *Antimicrob Agents Chemother*. 2012;56: 2987–2993.
28. D'Avolio A, Ciancio A, Siccardi M, et al. Inosine triphosphatase polymorphisms and ribavirin pharmacokinetics as determinants of ribavirinassociate anemia in patients receiving standard anti-HCV treatment. *Ther Drug Monit*. 2012;34:165–170.
29. Rau M, Stickel F, Russmann S, et al. Impact of genetic SLC28 transporter and ITPA variants on ribavirin serum level, hemoglobin drop and therapeutic response in patients with HCV infection. *J Hepatol*. 2013;58:669–675.
30. Ochi H, Maekawa T, Abe H, et al. ITPA polymorphism affects ribavirin-induced anemia and outcome of therapy—a genome-wide study of Japanese HCV patients. *Gastroenterology*. 2010;139:1190–1197.
31. Tanaka Y, Kurosaki M, Nishida N, et al. Genome-wide association study identified ITPA/DDRGK1 variants reflecting thrombocytopenia in pegylated interferon and ribavirin therapy for chronic hepatitis C. *Hum Mol Genet*. 2011;20:3507–3516.
32. Ahmed WH, Furusyo N, Zaky S, et al. Pretreatment role of inosine triphosphate pyrophosphatase polymorphism for predicting anemia in Egyptian hepatitis C virus patients. *World J Gastrolenterol*. 2013;19: 1387–1395.
33. Nemr N, Kishk R, Mandour M. Role of ITPA gene polymorphism in ribavirin-induced anemia and thrombocytopenia in Egyptian patients with chronic hepatitis C. *Indian J Gastroenterol*. 2016;35:7–13.
34. Sheikh AA, Humaira M, Zaki M, Bawani MA, Memon S. Severity of anemia during interferon and ribavirin therapy in patients with chronic active hepatitis C genotype-3 and its association with risk factors. *JLUMHS*. 2013;12:3.
35. Scherzer TM, Stättermayer AF, Stauber R, et al. Effect of gender and ITPA polymorphisms on ribavirin induced anemia in chronic hepatitis C patients. *J Hepatol*. 2013;59:964–971.
36. Miyamura T, Kanda T, Nakamoto S, et al. Roles of ITPA and IL28B genotypes in chronic hepatitis C patients treated with peginterferon plus ribavirin. *Viruses*. 2012;4:1264–1278.
37. Reau N, Hadziyannis SJ, Messinger D, Fried MW, Jensen DM. Early predictors of anemia in patients with hepatitis C genotype 1 treated with peginterferon alfa-2a (40KD) plus ribavirin. *Am J Gastroenterol*. 2008;103:1981–1988.
38. Shiffman ML, Salvatore J, Hubbard S, et al. Treatment of chronic hepatitis C virus genotype 1 with peginterferon, ribavirin, and epoetin alpha. *Hepatology*. 2007;46:371–379.
39. Asselah T, Moreno C, Sarrazin C, et al. Efficacy of a 12-week simeprevir plus Peginterferon/Ribavirin (PR) regimen in treatment-naïve patients

with hepatitis C virus (HCV) genotype 4 (GT4) infection and mild-to-moderate fibrosis displaying early on-treatment virologic response. *PLoS ONE*. 2017;12:e0168713. Available online at: https://dx.doi.org/10.1371/journal.pone.0168713.

40. Morio K, Imamura M, Kawakami Y, et al. ITPA polymorphism effects on decrease of hemoglobin during sofosbuvir and ribavirin combination treatment for chronic hepatitis C. *J Gastroenterol*. 2016. [Epub ahead of print].
41. Asselah T, Zeuzem S, Soriano V, et al. ITPA genotypes predict anemia but do not affect virological response with interferon-free faldaprevir, deleobuvir, and ribavirin for HCV infection. *PLoS ONE*. 2015;10:e0144004.
42. HCV Guidance: Recommendations for Testing, Managing, and Treating Hepatitis C. Available online at: https://www.hcvguidelines. org/full-report-view

How to cite this article: El Raziky M, Zayed NA, Abdel Baki A, Mansour SA, Shahin RMH. ITPA gene polymorphism (*94C>A*) effects on ribavirin-induced anemia during therapy in Egyptian patients with chronic hepatitis C. *J Med Virol*. 2017;89:1823–1829. https://doi.org/10.1002/jmv.24844