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LETTER TO THE EDITOR

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The role of ITPA and ribavirin transporter genes polymorphisms in prediction of ribavirin-induced anaemia in chronic hepatitis C Egyptian patients

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Summary

Few data are available concerning the roles of polymorphisms of inosine triphosphatase (ITPA) gene and ribavirin (RBV) transporter genes in the prediction of RBV-induced anaemia among Egyptians with chronic hepatitis C (CHC). Genotyping of three ITPA gene variants and two variants of RBV transporter genes has been performed in 123 patients under pegylated interferon-α/ribavirin treatment. The baseline haemoglobin and *ITPA rs1127354 CA/AA* have been found as predictors of anaemia at 4, 8 and 12 weeks of RBV therapy. In addition, *ITPA rs7270101 AC/CC* and age predicted anaemia after 12 weeks of therapy. In conclusion, the *ITPA* variant *rs1127354C>A* significantly predict RBV-induced anaemia during the first 3 months of treatment and it is recommended to be assessed before RBV administration.

KEYWORDS
anaemia, *ITPA*, pharmacogenetics, ribavirin, *SLC28A3*, *SLC29A1*

To the Editor:

Egypt has the highest prevalence of hepatitis C virus (HCV), particularly genotype 4 of the virus.[1] Until recently, the standard treatment for HCV genotype 4 infections was pegylated interferon α plus ribavirin (pegIFNα/RBV).[2] Despite the approval of many new direct-acting antivirals (DAAs),[3,4] still RBV is used as a part of modern HCV therapy where it improves sustained viral response (SVR) rates.[3]

Haemolytic anaemia is a frequent side effect of RBV and is a dose limiting factor that could potentially compromise SVR.[4,5] The identification of several host predictive factors that could influence RBV anaemia is required.[5]

Significant association between non-functional *ITPA* gene variant *rs6051702* and RBV-induced haemoglobin (Hb) reduction at week 4 has been reported. The association is attributed to the presence of two functional variants (*ITPA rs1127354* and *rs7270101*) that decrease *ITPA* activity in co-segregation with the protective *ITPA rs6051702* allele.[6] The predictive performance of RBV transporters in relation to RBV-induced anaemia has been considered. Significant association of transporter genes *SLC28A3 rs10868138T>C*[7] and *SLC29A1 rs760370 A>G*[8] with RBV-induced anaemia was reported in some studies, but not in others.[9,10]

Our study evaluated RBV-induced anaemia in the light of genetic polymorphisms of *ITPA rs6051702*, *ITPA rs1127354*, *ITPA rs7270101*, *SLC28A3 rs10868138* and *SLC29A1 rs760370* among a group of chronic hepatitis C (CHC) Egyptian patients.

A total of 123 treatment naïve adult Egyptian patients with CHC were included in the study and assessed in the period between July 2012 and October 2014. During this period, the standard protocol of treatment was pegIFNα-2a or pegIFNα-2b plus weight based RBV. The study was approved by the Ethics Committee (IRB00008718) of Assiut University. Written consent was obtained from participants. Inclusion and exclusion criteria were similar to other studies.[11,12] In addition, no treatment with growth factor before week 12 was added to inclusion criteria.

A blood sample was obtained from each patient and DNA was extracted using the QIAamp DNA Mini Kit. The allelic discrimination analysis was performed using the TaqMan assays. The analyzed SNPs were *ITPA rs727010A>C*, *ITPA rs6051702A>C*, *ITPA rs1127354C>A*, *SLC28A3 rs10868138* and *SLC29A1 rs760370 A>G*.

Delta Hb was taken as the clinical endpoint at weeks 4, 8 and 12. The term delta represented the difference between the value at a specific time and the value at the baseline. Patients were classified as anaemic when the absolute Hb value was lower than 10 g/dL or Hb reduction was more than 3 g/dL.

Statistical analyses were conducted using the SPSS ver. 20.0 (Chicago, IL, USA). Continuous variables were described as median values with the interquartile range. Categorical variables were described by frequency and percentage. Categorical data were compared using a Mann-Whitney statistical test. Any predictive power of the considered variables was evaluated by regression analyses: factors with a *P*-value<.2 in univariate regression analysis were considered in the multiple regression analysis.

General characteristics of the study patients are presented (Table 1). Minor allele frequencies (MAF) of *ITPA rs6051702A>C*, *ITPA rs1127354C>A* and *ITPA rs7270101A>C* were 15.9%, 5.7%, and 11.8% respectively. MAFs of RBV transporter genes *SLC29A1 rs760370A>G* and *SLC28A3 rs10868138T>C* were 41% and 6.1% respectively. All the studied SNPs were in Hardy-Weinberg equilibrium.

At week 4, *ITPA rs6051702 A>C* polymorphism showed a significant association (*P*=.012) with Hb reduction. The *AC/CC* group showed less delta Hb level (median value was −1.2 g/dL; IQR −1.9 to −0.35) reflecting more protection compared to homozygous *AA* with which Hb reduction was −1.9 g/dL (IQR −2.73 to −0.68). At week 8, *ITPA rs1127354* polymorphism was significantly related to delta Hb levels (*P*=.019). The median value of Hb reduction in *ITPA rs1127354 CA/AA* was −1.1 g/dL (IQR −2.15 to 0.2 g/dL) compared with the *CC* group where the reduction was −2.1 g/dL (IQR −3.25 to −1.1 g/dL). To detect factors that are able to predict anaemia univariate regression analysis and multiple regression analysis were performed at weeks 4, 8 and 12 and the results were presented (Table 2).

Reduction in Hb is significantly less at week 4 (*P*=.012) in the presence of *ITPA rs6051702 AC/CC* genotypes. The co-segregation of functional ITPA variants; *ITPA rs1127354* and *ITPA rs7270101*, known to reduce the activity of the ITPA enzyme with the non-functional variant *ITPA rs6051702;* explains the protective effect obtained against RBV-induced anaemia.[6]

The association between *ITPA rs1127354* variant and Hb reduction at week 8 of RBV treatment is significant (*P*=.019) compared with that at week 4 (*P*=.07). Similar results were previously reported in Egyptians.[13] A possible explanation is that at week 8 there is more Hb reduction than at week 4 that may be related to the higher RBV levels[14] which allows higher intracellular drug levels and increases the possibility of haematological toxicity.[8]

At week 12 none of the analyzed SNPs of *ITPA* is significantly associated with Hb reduction in our patients. However, a previous Egyptian study reported a significant association between *ITPA rs1127354* and percentage of Hb reduction at week 12. The discrepancy in results between the two Egyptian studies may be ascribed to the use of delta Hb in our study but the percentage of Hb reduction in the other Egyptian study.[15]

Although *ITPA rs7270101* is polymorphic in Egyptians, *ITPA rs7270101 AC/CC* genotypes are not significantly associated with Hb reduction throughout the 12 weeks; a finding that agrees with a previous Egyptian study.[16]

The results of the present study show that the *ITPA rs1127354* is an independent predictor of anaemia in Egyptian patients during the first 3 months of treatment. In addition, *ITPA rs7270101* is an independent predictor of anaemia at week 12 only. The data concerning *ITPA rs1127354* confirm previous reports[13,15,16] about the significant role of this polymorphism among the Egyptians.

TABLE 1 General characteristics of the study population

Characteristics	
Number of patients (n)	123
Median age (IQR) (years)	37 (27-45)
Male sex [n (%)]	106 (86.2)
BMI (kg/m^2) (IQR)	25.8 (23.4-29.1)
Median HCV RNA (log IU/mL) (IQR)	5.33 (4.48-5.76)
Median white blood cells (1000 cell/mm^3) (IQR)	5.4 (4.7-6.7)
Median platelet count (1000 cell/mm^3) (IQR)	219.5 (183-253.5)
Diabetes [n (%)]	1 (0.8)
Median ALT (IU/L)	17.6 (IQR 13.43; 22.3)[a]
METAVIR score [n (%)]	
F1	72 (58.5)
F2	34 (27.6)
F3	16 (13)
Median Hb levels (g/dL) (IQR)	
Baseline	13.8 (12.8-14.9)
Week 4	12.2 (11.5-12.9)
Week 8	11.7 (10.9-12.5)
Week 12	11.8 (10.8-12.5)
Anemia [n (%)]	
Week 4	21 (17.1)
Week 8	37 (30.1)
Week 12	38 (30.9)
Median Hb reduction (g/dL) (IQR)	
Week 4	−1.6 (−2.7 to −0.6)
Week 8	−2.0 (−3.1 to −1.0)
Week 12	−2.2 (−3.2 to −1.0)
Initial RBV dose [n (%)] (mg/d)	
1200	59 (48)
1000	52 (42.3)
800	12 (9.8)
Peg IFN type [n (%) and dose]	
α2a	62 (50.4) (180 μg/wk)
α2b	61 (49.6) (1.5 μg/kg per week)

ALT, alanine aminotransferase ([a]Upper limit of normal of ALT [ULN] = 12 IU/L); BMI, body mass index; Hb, hemoglobin; HCV, hepatitis C virus; IQR, interquartile range; Peg IFN, pegylated interferon; RBV, ribavirin.

TABLE 2 Univariate regression and multiple logistic regression analysis for detecting factors able to predict anemia after 4, 8 and 12 weeks

	Week 4 anemia		Week 8 anemia		Week 12 anemia	
	Univariate regression	Multiple regression	Univariate regression	Multiple regression	Univariate regression	Multiple regression
Variable	*P*; OR (IC 95%)	*P*; OR (IC 95%)	*P*; OR (IC 95%)	*P*; OR (IC 95%)	*P*; OR (IC 95%)	*P*; OR (IC 95%)
CNT3 rs10868138 TC/CC	.769; −0.236 (0.163; 3.820)		.457; −0.508 (0.158; 2.296)		.679; 0.246 (0.398; 4.110)	
ENT1 rs760370 GG	.532; 0.391 (0.434; 5.042)		.434; −0.473 (0.191; 2.039)		.809; 0.132 (0.393; 3.307)	
ITPA rs1127354 CA/AA	**.999**; −19.759 (0.000; NO upper limit written)	**.998**; −20.357 (0.000; NO upper limit)	**.096**; −1.764 (0.021; 1.369)	**.027**; −2.662 (0.007; 0.743)	**.089**; −1.805 (0.021; 1.313)	**.036**; −2.488 (0.008; 0.855)
ITPA rs7270101 AC/CC	.219; −0.960 (0.083; 1.770)		.546; −0.313 (0.264;2.021)		**.040**; −1.342 (0.073; 0.938)	**.027**; −1.625 (0.047; 0.828)
ITPA rs6051702 AC/CC	**.066**; −1.423 (0.053; 1.099)	.218; −1.047 (0.066; 1.854)	.394; −0.396 (0.721; 1.673)		**.164**; −0.669 (0.200; 1.313)	.682; 0.268 (0.362; 4.723)
Age	.859; 0.004 (0.959; 1.051)		**.122**; 0.031 (0.992; 1.074)	.136; 0.038 (0.988; 1.092)	**.099**; 0.033 (0.994; 1.075)	**.048**; 0.048 (1.000; 1.100)
Sex	.534; 0.493 (0.345; 7.769)		.238; 0.790 (0.593; 8.183)		.887; 0.082 (0.354; 3.330)	
BMI	**.088**; 0.145 (0.978; 1.365)	.113; 0.163 (0.962; 1.440)	.313; 0.068 (0.938; 1.220)		.501; 0.045 (0.918; 1.190)	
PegIFNα type	.449; 0.366 (0.559; 3.720)		**.003**; 1.237(1.508; 7.866)	.054; 1.001 (0.982; 7.541)	**.018**; 0.962 (1.180; 5.804)	.573; 0.283(0.496;3.549)
RBV dose	**.075**; 0.004 (1.000; 1.008)	.813; −0.001 (0.991; 1.007)	.250; 0.002 (0.999; 1.005)		.462; 0.001 (0.998; 1.004)	
Baseline Hb	≤**.001**; 0.891 (1.545; 3.849)	≤**.001**; 1.000 (1.673; 4.418)	≤**.001**; 0.879 (1.654; 3.504)	≤**.001**; 1.045 (1.793; 4.512)	≤**.001**; 0.653 (1.379; 2.675)	≤**.001**; 0.810 (1.509; 3.352)
Baseline WBCs	.247; −0.182 (0.613; 1.134)		.811; 0.027 (0.821; 1.286)		.837; 0.023 (0.819; 1.279)	
Baseline platelet	.490; −0.003 (0.987; 1.006)		.277; −0.004 (0.988; 1.003)		.619; −0.002 (0.991; 1.006)	

BMI, body mass index; Hb, hemoglobin; IC, interval of confidence; OR, odds ratio; PegIFN, Pegylated interferon; RBV, ribavirin; WBCs, white blood cells. Values in bold indicate significant results.

Ribavirin transporters play a definite role in intestinal absorption of the drug and its entry into the erythrocytes. Consequently, polymorphism of transporter-encoding genes could influence RBV pharmacokinetics and modify its clinical effects including anaemia.[7,17]

No association is present between either *SLC28A3 rs10868138* or *SLC29A1 rs760370* polymorphisms and anaemia in our study. These findings coincide with some studies[9,10] but not with others.[7,8] This discrepancy may be attributed to age differences of the selected patients and or the different definitions of anaemia among the different studies.

Further research is needed on a larger number of patients in whom RBV is included in the protocol of therapy to establish a schedule for RBV dose modification based on *ITPA rs1127354* genotype as a predictor of RBV-induced anaemia. It is important to consider also SVR when talking about RBV dose modification.[17,18] RBV-monophosphate concentrations in erythrocytes are related to anaemia and SVR in Sofosbuvir/RBV regimens.[19] On the other hand, RBV dose reduction due to anaemia in patients exposed to a drug regimen of ombitasvir-paritaprevir-ritonavir and dasabuvir plus RBV does not influence the SVR.[4] Accordingly, it has been hypothesized that RBV dose reduction will not influence SVR when the drug is given in combination with at least two potent new DAAs, compared to pegIFN/RBV or Sofosbuvir/RBV regimens.[4]

In conclusion, pharmacogenetic analysis of *ITPA rs1127354* could useful to predict RBV-induced anaemia during the first 3 months of treatment among Egyptian patients. This might help to adjust the RBV dose accordingly.

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DISCLOSURE

The authors disclose no conflicts of interest.

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