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Protective role of *ITPA* rs1127354-CA polymorphism against anemia in HCV patients using sofosbuvir ribavirin therapy: age and gender match case-control study

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Abstract

Background: Hepatitis C virus is affecting around 80 million people. Sofosbuvir ribavirin-based therapy is associated with certain side effects, especially anemia. Inosine triphosphatase (*ITPA*) genetic polymorphisms cause functional impairment in *ITPase* enzyme, leading protection against anemia and improving sustained viral response. This study aims to explore the impact of *ITPA* variants on hemoglobin decline, ribavirin dose reduction, and sustained viral response (SVR) achievement.

Methods: This is prospective gender and age matched case-control study of HCV genotype-3a infected individuals taking sofosbuvir-ribavirin treatment. Patient CBC, viral RNA, liver function test, and ribavirin dose reduction were recorded monthly. *ITPA*polymorphisms-rs1127354 were determined and confirmed by restriction fragment length polymorphism and sanger sequencing. Effects of polymorphism on hemoglobin level, ribavirin dose and treatment outcome were analyzed.

Results: *ITPA* rs1127354-CC genotype patients experience significant reduction in level of Hb leading to ribavirin dose reduction. Low mean Hb levels were observed in these individuals at first and last month of treatment. No statistical difference was observed in adverse effects on basis of *ITPA* genotype except fever. Age, BMI, and *ITPA* genotype rs1127354-CC were independently associated ($p< 0.05$) with a decrease in Hb level ⩾ 2g/dl below the baseline and ribavirin dose reduction. All patients with rs1127354 CA-genotype achieve SVR.

Conclusion: Pretreatment determination of *ITPA* polymorphism can further optimize HCV treatment with new direct-acting antivirals. *ITPA* rs1127354-CA has a protective role against ribavirin-associated anemia development and individualized management of ribavirin dose and along with the achievement of better SVR rates.

Introduction

HCV infection is globally prevalent chronic infections affecting about 354 million people [1]. The Hepatitis C virus is an enveloped, single-stranded RNA virus, belonging to the *Flaviviridae* family of viruses [2]. HCV genome contains a hypervariable region which subsequently results in multiple genotypes and sub-genotypes distributed across the world [3].

Globally Pakistan is ranked the second number after Egypt among countries with approximately 11 million HCV reported cases and continuously increasing HCV burden [1,4]. Unfortunately, HCV is much diverse in structure due to the high mutation rate, there is no strict pan-genotypic treatment available for the hepatitis C virus [5,6]. Treatment of HCV was revolutionized in mid-2011 by introduction of direct-acting antiviral therapy agents (DAAT) with much-improved treatment outcomes [7]. For past 20 years, ribavirin worked as an essential component of anti HCV antiviral therapy. It helped to improve treatment effectiveness but it causes therapy-limiting adverse effects. Initially, due to discovery of direct-acting antivirals (DAAs) it was anticipated that use of ribavirin as antiviral treatment would be abolished but in contrast to this ribavirin retains its role in the treatment and is particularly helpful for difficult-to-treat cases [8].

Polymorphism of the *ITPA* gene has been reported to predict anemia and treatment response while on therapy [9-11]. Deficiency of *ITPase* enzyme not only protect the individuals from anemia due to ribavirin but also help them to stick to their recommended and planned ribavirin dose [12].

Certain host-related pretreatment characteristics such as patient age, gender, and genetic polymorphisms are also reported to impact HCV antiviral response, disease progression, and treatment outcome by many researchers. As Asians seem to achieve better SVR rates as compared to Caucasians. The age of the patient and female gender was also reported to impact HCV therapy outcome [13-15]. Many researchers reported pretreatment demographic and baseline laboratory parameters to be connected with the achievement of sustained viral response. [16-18]. Narciso et al stated that gender, age, and pretreatment viral load have also been reported as independent prognostic factors for the achievement of sustained viral load in HCV-positive patients [17].

Studies from Japan reported an association of *ITPA* SNP rs1127354 polymorphism with anemia occurrence and reduction in the amount of ribavirin dose. They found baseline variables including the age of the patient, baseline Hb, and *ITPA* rs1127354-CC were independently associated with anemia development [10,19]. Less Hb reduction was observed in *ITPA* rs1127354 minor allele-A. Female experiences more Hb reduction. The study exhibited that being young and fertile age female is an independent factor which also play protective role against the development of ribavirin-induced hemolytic anemia [20].

Literature shows that patient genetic and demographic factors play etiological roles in ribavirin-associated anemia development and ribavirin dose reduction during ribavirin-based antiviral treatment. However, there is a paucity of published data on the risk factors linked with ribavirin-associated anemia in the Pakistani population. Therefore, the objective of this study was to explore the protecting role of *ITPA* CA genotypes on anemia due to ribavirin, ribavirin dose reduction, and therapy outcomes in HCV-infected individuals taking sofosbuvir ribavirin therapy, along with age and gender-matched *ITPA*-CC genotype patients (Controls) to reduce the confounding effects.

Methods

Study design and patient selection

Over-all 78 HCV infected patients with matched ages and gender were selected for the study. Patients were in the age range of 20-60 years, both genders, HCV genotype 3a infected, and were new for treatment. The patients were enrolled and drug sofosbuvir 400mg once daily along with weight-based ribavirin (100mg/day for <75Kg weight and 1200 mg/day for >75Kg body weight) was given for 6 months. After taking approval from the ethical committee reference number(IRB-UOL-FAHS/565) the HCV patients receiving direct-acting antiviral treatment were invited to participate after taking consent. Predesigned proforma was used to record the demographic data of HCV patients.

Efficacy assessments

At baseline, study samples were screened for Hepatitis C virus (HCV), Human immune deficiency virus (HIV) and Hepatitis B virus (HBV) by enzyme-linked immunosorbent assay (ELISA). Liver ultrasound and tests including real-time PCR Hepatitis C Viral RNA level, Liver function tests (LFTs) and Complete blood count (CBC) were performed monthly (1-6 months).

Study Endpoints

Individuals with the undetectable viral RNA at the end of 6-month antiviral therapy were considered to have achieved complete viral response (CVR). Undetectable HCV RNA at the end of 6-month treatment and detectable HCV RNA during follow-up was defined as Relapse. Patients with an undetectable viral RNA at the end of 6-month antiviral therapy and again 6 months after completion of therapy were considered to have achieved sustained viral response (SVR) [21,22]. Rate of SVR achievement was recorded Per protocol analysis

(PPA) in which those patients were included who adhere to therapy till completion and had SVR status assessed post-treatment completion [23]. Another important study endpoint was anemia in HCV patients during DAA (sofosbuvir ribavirin) therapy. Where anemia was defined as Hb concentration < 10.0 g/dL or a decrease in Hb concentration by > 2.0 g/dL from baseline Hb[19].

ITPA polymorphism determination through PCR-RFLP

Blood was drawn in aseptic conditions. DNA extraction was performed following the method previously described [24]. *ITPA* gene was amplified by following conditions: PCR master mix (Thermo scientific by Thermo fisher) was used with 5-10 ng/µl DNA. The primer sequence was used as mentioned earlier [23]. The thermal cycle conditions were: Denaturation at 94°C for 10 min then 35 cycles of 95 °C for 1 min, annealing at 55 °C for 30 sec, extension at 72 °C for 30 sec, and final extension at 72 °C for 10 min.

PCR products were visualized under ultraviolet light after running gel electrophoresis. PCR product band size was 213bp. Following PCR, *ITPA* genotypes were investigated using Restriction fragment length polymorphism (RFLP). For this purpose, PCR products were treated overnight with restriction enzyme Xcel to investigate the presence of rs1127354 polymorphisms. In the case of polymorphism rs1127354, 213bp PCR product was digested into fragments 213bp, 135bp, and 78bp for (CC) homozygous dominant allele, (CA) heterozygous allele, and (AA) for homozygous recessive allele respectively[23]. SNPs conformed to the Hardy-Weinberg equilibrium. For further confirmation of RFLP results, Sanger sequencing was conducted.

Patient allocation to groups

HCV patients were divided in two groups on basis of *ITPA* rs1127354 genotypes.

Group A= HCV-infected patients with *ITPA* genetic polymorphism rs1127354 genotype-CC.

Group B = HCV-infected patients with *ITPA* genetic polymorphism rs1127354 genotype-CA.

In both groups, there were an equal number of male and female patients and their age was also matched.

Statistical analysis

Comparative analysis of data was performed concerning the *ITPA*rs1127354 genotype of the patient. Data normality was tested using Shapiro-Wilk test. Qualitative variables were reported as percentages and frequencies whereas quantitative variables were reported as interquartile range (IQR), mean ± standard deviation (SD) and medians. Chi-square test (X2) was used to compare categorical variables. Continuous variables were analyzed through Mann-Whitney U test. Log-rank test and Kaplan-Meier analysis were used to determine and compare ribavirin dose reductions between both groups (A and B) on basis of *ITPA* rs1127354 CC and CA genotypes. Binary logistic regression was applied to identify variables associated with Hb reduction ≤ 2g/dl during 6-month treatment. A *p*-value of less than *0.05* was considered statistically significant. All tests were two-tailed and performed using the SPSS software version 25.

Results

Over all 78 HCV infected individuals were enrolled for the study out of which 68 (divided in 2 groups on basis of *ITPA* rs1127354 CC and CA genotype) patients completed 6-month antiviral therapy. The *ITPA* rs1127354 major allele-C frequency was 0.875/78 and minor allele-A frequency was 0.125/78. Forty-four (64.7%) out of 64 patients were males and 24(35.3%) were females. Each group (group A *ITPA* rs1127354-CC) and (group B *ITPA* rs1127354-CA) contain 34 patients with 24 males and 15 females. Normal liver architecture was seen in 50% of patients whereas 28 (41.2%) patients were having fatty liver. No significant difference ($p<0.05$) was observed between the liver architecture of patients on basis of *ITPA* SNP's. Also, no statistical evidence of difference in pretreatment characteristics of both (group-A and group-B) patients was observed (Table 1).

Variable	Group A CC genotype	Group B Non-CC genotype	*p*-value
Age	42.4±12.0 (21-59)	42.4±12.4(21-59)	0.95
BMI	24.7±5.0 (16.7-37.1)	25.3±3.8(20-34)	0.49
Hemoglobin g/dl	13.0±1.0(11-15.9)	13.1±1.3(11.8-16.1)	0.52
HCV RNA by PCR (IU/ml)	2.6 $X10^6$±5.6 $X10^6$ (1.3 $X10^4$-3.2 $X10^7$)	2.7 $X10^6$±3.9 $X10^6$ (1.3 $X10^4$-1.3 $X10^7$)	0.81
Hematocrit l/l	38.7±3.1(34-46)	38.6±3.7(35-49)	0.94
Total leukocyte counts (IU/L)	6.9±2.1(4-15.5)	7.8±2.2(3.9-13.1)	0.16
Platelets 10^9 cells/L	219±69(100-400)	204±67(100-401)	0.23
Total Bilirubin (mg/dl)	0.7±0.2(0.4-1.5)	0.7±0.4(0.4-1.9)	0.40
ALT (IU/L)	73±43(25-225)	69±33(24-162)	0.62
AST (IU/L)	82±58(25-216)	72±45(29-201)	0.33
Serum Urea mg/dl	28.9±3.5(21-35)	28±4.8(21-39)	0.35
Serum creatinine mg/dl	0.7±0.1(0.6-1.4)	0.7±0.1(0.5-1.0)	0.59

Abbreviations: Alanine aminotransferase (ALT), Body mass index (BMI), Aspartate amino transferase (AST), Standard deviation (SD)

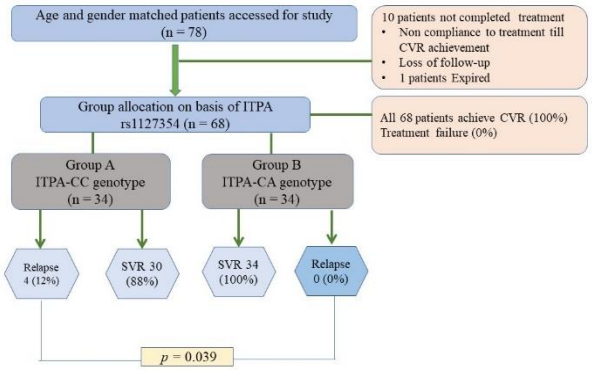
Table 1: Demographic profile of HCV infected individuals with reference to their *ITPA* rs1127354 CC and CA genotypes

ITPA rs1127354 genotypes and treatment outcome

Overall, CVR achievement rate was 100% as all patients were negative for HCV RNA presence after 6-month anti-HCV antiviral therapy. Sixty eight out of 78 patients, were positively followed till SVR per-protocol analysis (PPA). Overall SVR achievement rate was 94.1%, whereas the remaining showed relapse upon follow-up 6 months after treatment completion. There was no significant difference in SVR achievement rates on basis of patient gender ($p = 0.52$). However, there was significant difference in SVR achievement rates of

HCV-positive patients on basis of patient *ITPA* rs1127354 genotype. As all patients (100%) with the *ITPA* rs1127354 CA genotype achieve SVR with no case of relapse was observed. Figure 1 shows details of *ITPA* SNP rs1127354 wise distribution of treatment outcome.

In our study, 45.3% of infants weighed 1000 to 1500 g, 33.1% weighed more than 1500 g, and 21.6% weighed less than 1000 g. The results of patient characteristics, complications of prematurity and the need for oxygen and respiratory ventilation in the intervention and control groups are shown in Tables 1-3.



Abbreviation: Complete viral response (CVR), Sustained viral response (SVR), number of patients (n). Inosine triphosphate (*ITPA*).

Figure 1: Flow chart presents *ITPA* rs1127354 genotype wise distribution of anti-HCV antiviral treatment outcome (SVR achievement) in HCV patients receiving Sofosbuvir ribavirin therapy.

Reduction in Hb level and ribavirin dose on basis of *ITPA* rs1127354 genotypes

Hb reduction from baseline to the 1st month of treatment was recorded and 45 (66.2%) HCV patients experience >2g/dl Hb reduction from baseline to the first month of treatment and only 23(33.8%) patients presented < 2g/dl Hb reduction. Statistically significant difference ($p \leq 0.05$) was observed in the level of Hb reduction concerning baseline in *ITPA* rs1127354 figure 2 (A). Ribavirin doses were decreased in response to Hb reduction. Overall,41(60.3%) patients experience ribavirin dose reduction as compared to the planned ribavirin dose. Patients having *ITPA* SNP rs1127354-CC genotype frequently experienced ribavirin dose reduction in comparison to CA genotype figure 2 (B).

There was no statistical difference in mean hemoglobin levels of both groups A and group B patients at baseline. Monitoring of patients' hemoglobin levels on monthly basis presented a statistically significant difference ($p < 0.05$) in mean hemoglobin levels on the first and second month of therapy on basis of *ITPA* rs1127354 genotypes. A significant difference in mean hemoglobin levels was further found at the 5th and 6th months of treatment Table 2.

Throughout the treatment, less than 10 g/dl Hb reduction was observed in 21% of patients. Significant difference ($p= 0.01$) was found in an overall number of patients who experience Hb levels 10g/dl or below on basis of patients' *ITPA* genotype. Statistically significant difference ($p= 0.04$) was also found in hemoglobin levels of patients who had less than 10g/dl Hb at 1st month of treatment as compared to baseline hemoglobin levels on basis of *ITPA* rs1127354 genotypes CC and CA. HCV patients with *ITPA* rs1127354 -CC genotype frequently experience Hb less than 10g/dl at the first month of treatment (Figure 3).

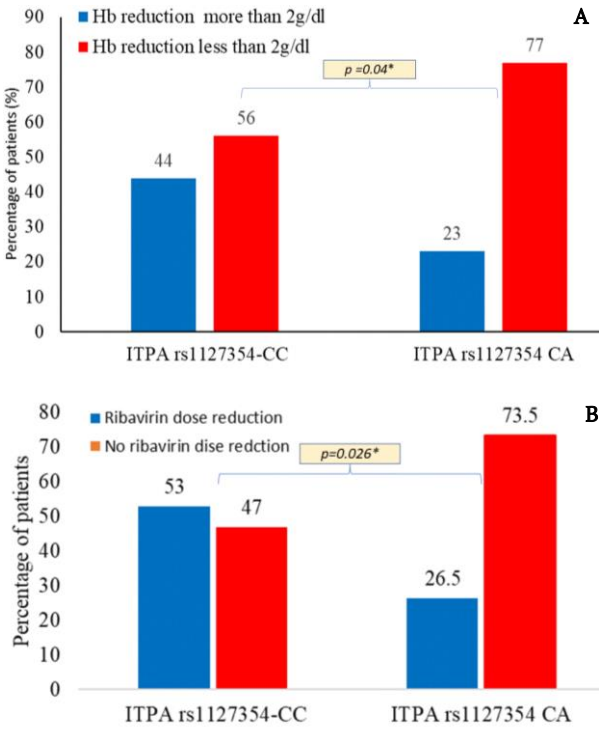


Figure 2: Reduction in Hemoglobin level and ribavirin dose reduction on basis of *ITPA* rs1127354 genotypes. (A) Hemoglobin decline ≥ 2g/dl from baseline to 1st month of treatment with reference to patients *ITPA* rs1127354 genotypes.(B) Ribavirin dose reduction during treatment with reference to patients *ITPA* rs1127354 genotypes. * Statistically significant *p value.*

Patient Hb month-wise	*ITPA* rs1127354				*p -Value*
	Group A CC genotype (n=34)		Group B CA genotype (n=34)		
	Mean ±SD	Range	Mean ±SD	Range	
Hb (BL)	12.9± 1.0	11.0-15.2	13.2 ± 1.3	11.9-16.1	0.523
Hb (1M)	11.1± 1.5	8.1-14.1	12.0 ±1.8	8.6-15.2	0.02 *
Hb (M2)	11.0± 1.3	7.9-13.9	11.8 ±1.4	9.4- 14.3	0.05 *
Hb (M3)	11.0 ±1.3	7.5-14.0	11.5 ± 1.2	9.5-14.5	0.23
Hb (M4)	11.0 ± 1.2	8.0-13.8	11.6 ±1.1	9.7- 14.6	0.12
Hb (M5)	10.9 ±1.1	8.6-13.8	11.5 ±1.1	10.0-14.3	0.03 *
Hb (M6)	10.8 ±1.2	8.6-14.1	11.4 ± 1.3	10.0- 14.2	0.04 *

Abbreviation: Hemoglobin (Hb), Month (M), M1, M2, M3, M4, M5, M6 presents time in months. Baseline (BL), Number of patients (n), Standard deviation (SD). * Statistically significant ($p < 0.05$).

Table 2: Month-wise comparison of hemoglobin reduction on basis of HCV infected patient's *ITPA* rs1127354 genotypes.

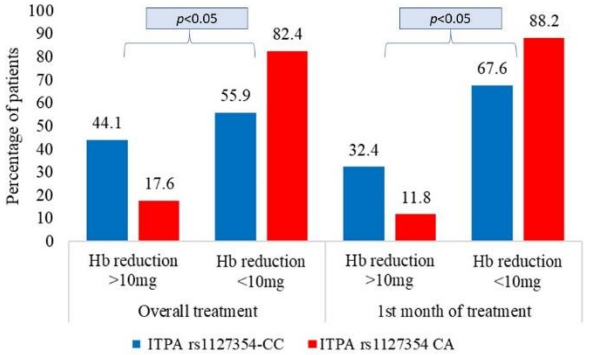


Figure 3: Proportion of HCV infected patient with ≤ 10g/dl Hemoglobin reduction overall treatment and at 1st month of treatment on basis of *ITPA* rs1127354 CC and CA genotypes.

Safety and tolerability analysis

HCV patients taking sofosbuvir ribavirin also experience different adverse effects during antiviral treatment. Patient-reported adverse effects were recorded and compared on basis of patient gender statistically significant difference ($p < 0.05$) was found in complaints of nausea and muscle pain where female patients reported these complaints significantly high ($p<0.05$). No statistical difference was observed in patients reported adverse effects concerning *ITPA* genotypes except fever which was reported in a significantly high ($p< 0.05$) number of individuals with *ITPA* rs1127354-CC genotype. The most commonly reported adverse effect was fatigue, fever, joint ache, and headache Figure 4.

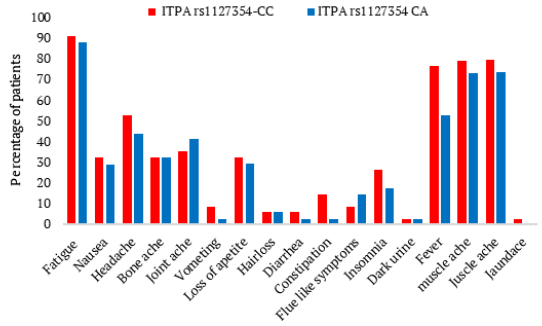


Figure 4: HCV infected patient reported adverse effects experienced during sofosbuvir ribavirin antiviral therapy. * Presents statistically significant *p value.*

Survival analysis

As a result of adverse effects like anemia, ribavirin dose was reduced in on-therapy chronic HCV-infected patients at different months of treatment. Survival analysis using a long-rank test was performed to determine the difference in the number of months when patient experience the 1stdose reduction of ribavirin with reference to *ITPA* rs1127354 CA and CC genotypes. A significant difference ($p = 0.04$) was observed between month-wise (baseline to 6-month therapy) ribavirin dose decrease in *ITPA* CC and CA genotype figure 5.

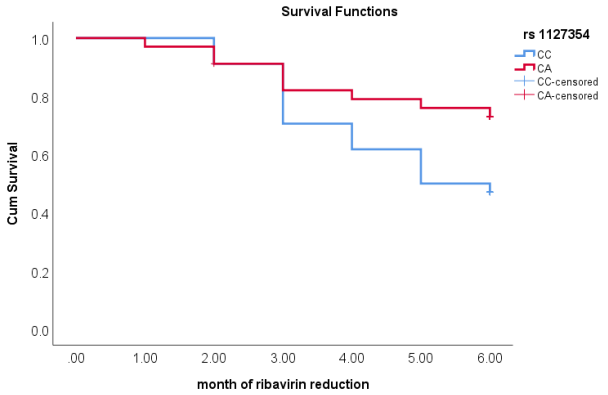


Figure 5: Kaplan-mere survival curve presenting ribavirin dose reduction on basis of *ITPA*CC and CA genotypes in HCV infected patients.

Predictive factors associated with Hemoglobin reduction ⩾2g/dl below the baseline Hemoglobin levels

To find out which patients' pretreatment characteristics can impact the decrease of hemoglobin level leading to ribavirin dose reduction Binomial logistic regression was applied Nagelkerke $R^2$was 57 %. Age of the patient, BMI, and *ITPA* genotype rs1127354-CC were significantly ($p<0.05$) linked with decrease in Hb level ⩾ 2g/dl below the baseline Hb value (Table 3).

Baseline variables	Odd ratio (95% CI)	*P-value*
Age	1.12(1.04-1.22)	0.003*
Gender	0.28(0.03-2.5)	0.26
BMI	0.79(0.63-0.98)	0.03*
ITPA rs1127354	7.13(1.3-37.4)	0.02*
Hb	1.02(0.44-2.37)	0.95
Viral load	1.00(1.00-1.00)	0.28
WBC	0.90 (0.61-1.33)	0.62
PLT	0.99(0.98-1.00)	0.26
Total bilirubin	0.62(0.04-8.1)	0.71
ALT	0.97(0.93-1.00)	0.10
AST	1.03(0.99-1.06)	0.08
Urea	0.88(0.72-1.07)	0.22
Creatinine	1.20(0.008-176)	0.94

Abbreviations: Hemoglobin (Hb), Body mass index (BMI), White blood cell count (WBC), alanine aminotransferase (ALT), Aspartate amino transferase (AST), Platelet (PLT).* Presents statistically significant *p-value.*

Table 3: Logistic regression analysis of HCV patient baseline characteristics associated with a reduction in Hb level ≥2g/dl below the baseline Hb value.

Discussion

HCV is a positive sense RNA virus that is an important cause of chronic hepatitis, cirrhosis, and liver cancer, affecting about 150 million individuals globally[25]. Understanding of HCV pathogenesis guided researchers in development of tolerable oral direct-acting antivirals. DAA therapies are more specific in action and can be used as single therapies or in combination with other therapies (interferon and ribavirin) with more than 90% HCV cure rates [5]. Researchers from

Pakistan stated that interplay between environmental, viral and host-based factors determine clinical outcome of hepatitis C infection. The study showed the relevance of different genetic polymorphisms in HCV-infected patients in response to treatment. They reported that pretreatment evaluation of host genetics can be used to upgrade anti-HCV therapies. Genotyping of Pakistani individuals for the relevant polymorphisms should be conducted tocategorize the individuals which are at greater risk of side effect development patients and those who can give a better therapy outcome. Genotyping of patients at baseline would have both financial and clinical benefits in the long term [26]. Based on previous literature, *ITPA* polymorphism has been identified to affect *ITPA* activity and reduce the expression of the *ITPA* gene causing *ITPase* deficiency and leading to ribavirin-induced anemia. The frequency of this polymorphism is reported different in different populations [27]. In south Asian populations the *ITPA* rs1127354 major allele-C frequency was 0.867/351 and the minor allele frequency was 0.132/351 [28]. Similar allele frequency was observed in the present study as *ITPA* rs1127354 major allele-C frequency was 0.875/78 and minor allele-A frequency was 0.125/78.

Current study presented a significantly high number of individuals with the *ITPA* rs1127354-CC variant experienced ⩾2g/dl Hb reduction as compared to patients having *ITPA* rs1127354-CA genotype when Hb levels were evaluated with reference to baseline Hb levels. Similarly, *ITPA* rs1127354-CC genotype patients frequently experience Hb less than 10g/dl at the first month of treatment as compared to the *ITPA* rs1127354-CA genotype. The frequency of the patients with ribavirin dose reduction was also significantly higher in the *ITPA* SNP rs1127354-CC genotype as compared to the CA genotype due to Hb reduction. Monitoring of patients' hemoglobin levels on monthly basis during HCV antiviral therapy presented *ITPA* rs1127354-CC patients had low mean Hb levels at different months especially at the first month of treatment and near the end of treatment. SVR achievement rate was significantly high ($p = 0.03$) 100% in *ITPA* rs1127354 CA patients with no case of relapse observed in rate *ITPA* rs1127354 CA patients. Consistently, a study from Japan has not found statistical evidence that variation in *ITPA* genotype directly associated with SVR, although in their study all non-SVR patients were *ITPA*-CC genotypes. Although their results indicated that *ITPA* variant rs1127354CA/AA plays a significant role in preventing ribavirin-induced severe anemia [29]. Another study on Japanese HCV genotype 2 infected individuals determined that *ITPA* CC genotype individuals had significantly higher Hb reduction and ribavirin dose reduction throughout treatment than those with a non-CC genotype. Overall Hb level below 10g/dl was also more frequently observed in *ITPA*-CC patients. Increased age of the patient and *ITPA*-CC genotype independently associated with significant Hb reduction throughout the treatment course. SVR achievement rate was 98.7% in the *ITPA*-CC genotype and 100% in non-CC genotype patients. They described that polymorphism of the *ITPA* gene seemed to correlate with incidence of anemia and ribavirin dose reduction during sofosbuvir ribavirin treatment, but not with treatment clinical outcome [30].

Sheikh et al. from Peshawar Pakistan also determined that chronic HCV-infected patients having CA/AA genotype at *ITPA* SNP rs1127354 are protected against anemia while receiving interferon and ribavirin combination therapy [31]. Similarly study from Hyderabad Pakistan evaluated anemia frequency (Hb level <10gm/dl) in HCV (genotype-3) infected individuals at 1st, 3rd and 6th months of ribavirin-based therapy. They experience that, anemia is a common obstacle of ribavirin-based anti-HCV treatment. Total, 76.4% of patients experiences more than 2 grams dropped in hemoglobin at 1st month from baseline and 22% of patients experienced anemia Hb<10 gm/dL. Anemia was more frequently observed in female HCV patients [32]. A meta-analysis also described significant association between *ITPA* rs1127354 genetic variation and Hbreduction, severe anemia, reduction in ribavirin, and SVR achievement rates of HCV-infected patients. Their results indicated *ITPA* rs1127354-CA genotype patients were protected to develop hemolytic anemia, severe anemia and showed good SVR achievement rates as compared to *ITPA* rs1127354-CC genotype patients and testing for *ITPA* genetic variations can benefit patients [33]. A study from Italy reported *ITPA* rs1127354 genetic variations in HCV-infected patients as a predictor of ribavirin-associated anemia overall and at the 1st month of treatment. They aimed to forecast anemia at 1st month, after which clinicians usually assess and readjust ribavirin dose. It was experienced that variant allele (CA/AA) in rs1127354 and Hb level at baseline was independently linked to protection against significant anemia at 1st month. They also study other *ITPA* polymorphisms rs7270101 and rs6051702 and they were also significantly associated with anemia onset in their population. Their study proposed the pretreatment identification of *ITPA* polymorphisms to estimate the individual risk of treatment-induced anemia and can have increased ribavirin dose in individuals with a smaller risk of anemia and improved SVR rates [34]. Researchers from Spin studied polymorphism *ITPA* rs1127354 and the risk of anemia due to ribavirin at the 1st, 3rd and 9th month of treatment with reference to baseline Hb in

HCV/HIV coinfected individuals receiving ribavirin with interferon as antiviral therapy. Hb reduction was significantly greater in patients with the *ITPA* rs1127354-CC genotype than in those with the CA/AA genotype. Ribavirin dose reduction and use of erythropoietin therapy were significantly high in *ITPA*-CC genotype patients. Data suggest the polymorphism of *ITPA* influence Hb levels, ribavirin dose reduction, and frequency of erythropoietin use but have no impact on SVR achievement in their population. In their study patients were infected with different HCV genotypes (1,3 and 4) [35]. A study from Iran stated that both host and virus-related parameters play an important part in hepatitis C progression and therapy response. Polymorphism of the *ITPA* gene influence ribavirin-based hemoglobin decline and is an appropriate candidate for predicting ribavirin-induced hemoglobin decline in the Irani population [36]. Assessment of anemia risk using patient *ITPA* genotyping is especially useful for high-risk patients such as patients with pre-existing anemia, elder age or lower renal function [29].

In present study no statistical difference in patient-reported adverse effects with reference to *ITPA* CC and CA genotypes was observed except fever which was reported in a significantly high ($p < 0.05$) number of individuals with *ITPA* rs1127354-CC genotype. The most commonly reported adverse effect was fatigue, fever, joint ache, and headache. Age of the patient, BMI, and *ITPA* genotype rs1127354-CC were significantly ($p< 0.05$) associated with a decrease in Hb level ⩾ 2g/dl below the baseline Hb value leading to ribavirin dose reduction. Researchers from Pakistan also described the influence of patient demographic factors such as age and gender impact the incidence of HCV and antiviral therapy treatment response [37-39]. Anti-HCV therapy is also associated with certain adverse effects such as hematological abnormalities influenza-like symptoms and psychiatric symptoms which could result in dose reduction or even discontinuation of therapy. To avoid these adverse events and reduce the cost of antiviral treatment it is essential to predict an HCV patient's response before treatment through evaluation of the patient's baseline characteristics [40]. The most frequently adverse events of direct acting antivirals in previous studies were fatigue, nausea, diarrheaand, headache[41-43]. Welzel et al. also reported that sofosbuvir ribavirin combination therapy is safe and effective as antiviral therapy for HCV patients. The adverse events commonly reported during treatment included anemia, fatigue, nausea, headache, rash, insomnia, and flu-like symptoms. About 2.8% of patients discontinue treatment due to adverse effects [44]. In contrast to the present study, Attia*et al.* reported that the males frequently reported occurrence of adverse events to DAA therapy [45]. Vidal et al. reported no significant associations between flu-like symptoms, depression, and *ITPA* genetic variants however gastrointestinal disturbances was associated with *ITPA* polymorphism (p=0.04) [46]. A study from Karachi Pakistan reported ribavirin dose reduction in around 16% of patients during anti-HCV antiviral therapy. Whereas weakness, light-headedness, and fatigue were frequently reported as adverse effects. Around 23.6% of patients had severe anemia. The anemia was significantly associated with the age of the patients (p<0.05) but not with gender [47]. Similarly, Thompson et al. reported that minor allele *ITPA* polymorphisms rs1127354 were associated with ribavirin-associated anemia but were not associated with an increase in SVR rate [48]. Kim et al. reported from Korea also reported similar findings [49]. Urabe et al reported individuals with the *ITPA* CC genotype experienced frequent Hb reduction during antiviral therapy than individuals with *ITPA* CA/AA genotype [50].

This age and gender-matched case-control study was conducted on two groups (*ITPA*-CC and CA) patients infected with HCV genotype 3a taking sofosbuvir ribavirin treatment to minimize the confounding effect of the difference in age, gender, HCV genotype, and therapy regimens. The study concludes that patients having the *ITPA* rs1127354-CC genotype are prone to on-therapy anemia development leading to ribavirin dose reduction, especially at the first month of treatment after which clinicians adjust the treatment dose. A statistically significant impact of patients' *ITPA* genotype rs1127354 CA was observed on the SVR achievement rate as all patients with *ITPA* rs1127354 CA genotype achieve SVR. However, no significant difference was recorded in patient reported adverse effects on basis of *ITPA* genotype difference except fever was reported in a significantly high number of patients with *ITPA* rs1127354-CC genotype. The study highlight, that patient age, BMI, and *ITPA* genotype rs1127354-CC were significantly associated with a decrease in Hb level ⩾ 2g/dl below the baseline Hb value leading to ribavirin dose reduction. These results also suggested the protective/ valuable role of *ITPA* rs1127354-CA genotype for individualized management of on-therapy anemia, ribavirin dose along with the achievement of better SVR rates. Pretreatment identification of patient *ITPA* genotype can work as a prognostic tool in clinical practice to identify patients with high-risk of anemia in management of HCV infection and those who can get the beneficial effect of ribavirin. Pretreatment determination of *ITPA* polymorphism can further optimize HCV treatment with new direct-acting antivirals.

The limitation of the study was a smaller sample size especially the number of female patients was less in both groups.

Future: Further studies determining the serum level of ribavirin and its association with Hb reduction can be done. The impact of the *ITPA* rs1127354 genotype on serum *ITPase* enzyme levels can be measured.

Competing Interest

The authors declare that there is no conflict of interest.

Author Contributions

Sameen Amjed:experimental work, Manuscript writing and data analysis

Hafiz Ghulam MurtazaSaleem, Sajjad Ullah, Shabana: conceptualized, supervised, Manuscript review and Data evaluation

Junaid Jafar: data analysis and technical support

Shahzad Latif: Clinical supervision and technical support

Ahmed Bilal Waqar: Manuscript scanning and correspondence

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