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Original Article

Association of Inosine Triphosphatase Polymorphysims rs7270101 and rs1127354 with the occurrence of Anemia in Hepatitis C patients receiving Pegylated Interferon and Ribavirin

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Abstract:

Background: HCV presents major health care problem in Pakistan. Hepatitis C patients if not well treated may progress to liver cirrhosis and cancer. Currently recommended directly acting antiviral drugs produces better cure rate and less adverse effects than earlier drugs peg-interferon and ribavirin. Peg-interferon and ribavirin combination therapy produces anemia in hepatitis C patients which is the main reason for treatment discontinuation or dose reduction. Anemia due to peg-interferon and ribavirin therapy is associated with ITPA polymorphism.

Objective: To find the association of ITPA polymorphisms rs7270101 and rs1127354 with occurrence of anemia in hepatitis C patients receiving peginterferon and ribavirin combination therapy.

Methods: DNA was extracted from blood samples of treatment naïve hepatitis C patients. Two SNPs of Inosine triphosphatase rs7270101 and rs1127354 were genotyped by means of allelic inhibition of displacement activity method. Complete blood count of the patients was carried out first before starting interferon and ribavirin therapy and then after three months of therapy.

Results: 20 out of 115 patients had more than 3gm/dl reduction in hemoglobin level after 3 months of anti-HCV therapy. At rs1127354, 69 patients showed the presence of CC genotype, 38 were found to have CA and 3 patients had AA genotype. The minor allele A at rs1127354 was found to be protective against anemia due to peg interferon and ribavirin combination therapy in hepatitis C patients (Odds ratio=0.275, C.I= [0.081-0.938], Chi2=4.77, p=0.02889) while the major allele C at rs1127354 was found to be associated with anemia in HCV patients receiving interferon and ribavirin combination therapy (Odds ratio=3.638, C.I= [1.066-12.409],Chi2=4.77, p=0.02889).At rs7270101 all the patients showed AA genotype.

Conclusion: The current study concludes that CA/AA genotype at ITPA SNP RS1127354 provides protection against anemia in hepatitis C patients receiving interferon and ribavirin combination therapy.

K**ey Words:** Inosine Triphosphatase (ITPA), Polymorphysims, Anemia, Hepatitis C, Interferon, Ribavirin

Introduction

About 2% of the population in world is hepatitis C virus (HCV) positive with almost 150 million people being affected with the disease.[1]

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Around 27% of hepatic cirrhosis and 25% of hepatocellular carcinomas occur in HCV infected patient.[2] Average prevalence of hepatitis C in Pakistan is approximately 4.7%.[3]

Depending upon the HCV genotype direct acting antiviral drugs such as sofosbuvir, glecaprivir, ledispravir etc. are currently recommended for the treatment of chronic hepatitis C (CHC) as they have better cure rate and less adverse effects compared to

earlier drugs peginterferon and ribavirin.[4,5] Among these adverse effects anemia is the most important that results in treatment discontinuation or ribavirin dose reduction in approximately 9-22% of the HCV infected patients [6,7]. Both interferon and ribavirin reduce hemoglobin level. Interferon damages bone marrow while ribavirin causes destruction of red blood cells (RBCs).[8] Ribavirin causes formation of ribavirin triphosphate (RBV-TP) in RBCs that causes red cell destruction. Ribavirin is involved in the removal of guanosine triphosphate (GTP) which decreases adenine triphosphate (ATP) level in human RBCs. Since oxidative metabolism of RBCs depend upon ATP, therefore decrease in ATP level in RBCs inhibits oxidative metabolism leading to premature lysis of RBCs.[9]

Studies on pharmacogenomics of HCV show that polymorphisms in IL28B and Inosine Triphosphatase (ITPA) are significantly related with the results of interferon and ribavirin therapy. IL28B polymorphisms gives an idea about the response of the individual to interferon and ribavirin treatment (10) while ITPA polymorphisms determine risk of anemia in these patients.[10,11]ITPase deficiency causes increased accumulation of ITP in RBCs which compensates GTP depletion by ribavirin. The accumulated ITP competes with RBV-TP and thus prevents premature destruction of R.B.Cs by decreasing toxic effects of RBV-TP.[9]Accumulation of ITP in red blood cells is beneficial because it competes with RBV-TP and thus inhibits the destruction of red blood cells. Studies show that ITPA deficiency is associated with less decrease in Hb level due to peg-interferon and ribavirin combination therapy .[7]

According to genome wide association studies (GWAS) two genetic variants of ITPA rs7270101 and rs1127354 are strongly associated with anemia due to interferon and ribavirin treatment.[12] These polymorphisms reduce the function of ITPA and increase the concentration of ITP in RBCs that provides protection against decrease in hemoglobin level.[13]

Literature lacks studies exploring effects of interferon on Hb levels, with reference to the polymorphisms discussed, in local settings. This research was, hence, conducted to elucidate the association between ITPA polymorphisms and decrease in Hb level due to interferon and ribavirin treatment in Pakistani patients.

Methods

This prospective study was conducted from March 2014 till August 2014 when interferon plus ribavirin combination therapy was the mainstay of treatment for CHC. Treatment naïve HCV positive patients belonging from district Dera Ismael Khan of Pakistan, presenting at Mufti Mahmood Teaching Hospital (MMTH), were enrolled into the study. Those with previous history of anemia or at risk of developing anemia due to any other active systemic or metabolic disorder were excluded from the study. Informed written consent was acquired from all the participants.

A comprehensive questionnaire encompassing the demographic and clinical details was filled out for each patient by a pre-trained physician. A volume of 6mL venous blood was collected in two EDTA coated sample collection tubes. Complete blood count (CBC) was determined on Sysmex KX-21®(Sysmex Corporation, Kobe, Japan) automated hematology analyzer according to manufacturer's guidelines. Pure Link Genomic DNA® Kits (Invitrogen, USA) were used to extract DNA from the blood samples of study subjects. Two SNPs rs7270101 and rs1127354 were genotyped by means of allelic inhibition of displacement activity method (13). Details of primers used for SNP genotyping are given in Table 1.

Table 1. Sequences of primers for ITPA genotyping

Primers	Sequences
rs7270101 F(A)	CACGTGCTCACATGGAGAA
F(B)	ACCGTATGTCTCTGTTTTGTTTTC
R(C)	CTACCTGGACAAGAAGAGCA
rs1127354 F(D)	AGAATTCCTGGTCTAGGAGGA
R(E)	TGCCACCAAAGTGCATGG
R(F)	CGAACTGCCTCCTGACAT

Optimization studies were carried out and conditions were adjusted for polymerase chain reaction (PCR) amplification of the SNP. Best results for primer annealing were obtained at 57°C for rs7270101 and at 55°C for rs1127354. Genotyping results for both the SNPs were analyzed by loading 10 µl PCR products on 2% agarose gel along with 4 µl of 6X loading dye. 100 or 50bp DNA ladder was also loaded with the DNA samples for size discrimination. Gel was run at 90 volts for 60 minutes and the results were visualized under UV.

The patients were followed at three months interval, another blood sample of 3mL volume was obtained and CBC was repeated on the same machine as used previously at MMTH. Patients with Hb reduction ≥3g/dL and<3g/dL after 3 months of interferon and

ribavirin combination therapy were segregated as Group-1 and Group-2, respectively.

Percentage frequencies of genotypes and alleles were calculated using Microsoft Excel® 2010. Polymorphisms were tested for deviation from Hardy*Weinberg equilibrium by means of Chi*square test using online program OEGE*online encyclopedia for genetic epidemiology studies. Odds ratio and 95% confidence interval were calculated for genotypes and alleles in order to determine their effect size in our population. This was done using online software programs MedCalc and Contingency table.

Results

It was found that out of total patient population of 115, 61 (53.04%) were males and54 (46.95%) were females with an age range of 19-50 years. Among the study patients, 20 (17.39%) were found to have suffered significant post-treatment decrease in Hb level and were hence place into Group-1; leaving out 95 (82.60%) patients in the Group-2. Among the Group-1 patients, 12 (19.67%) were males, whereas 8 (14.81%) were females. Mean post-treatment Hb decline,among the study patients (n=115), was found to be 3.36mg/dL in Group-1 patients and 0.805 in Group-2patients. The characteristics of study population are summarized in Table 2.

Table 2.Characteristics of study population (n=115)

Characteristics	Group-1*	Group-2**
Number	20	95
Sex (Male/Female)	12/8	49/46
Age (years)	36±7.6	34.83±9.613
Pretreatment Hb (g/dL)	14.915±1.0839	13.105±1.071
Hb at week 12 (g/dL)	11.55±0.976	12.321±1.071
Hb reduction (g/dL)	3.365±0.539	0.805±0.483

*Patients with post-treatment Hb decrease ≥3g/dL
**Patients with post-treatment Hb decrease ≤3g/dL

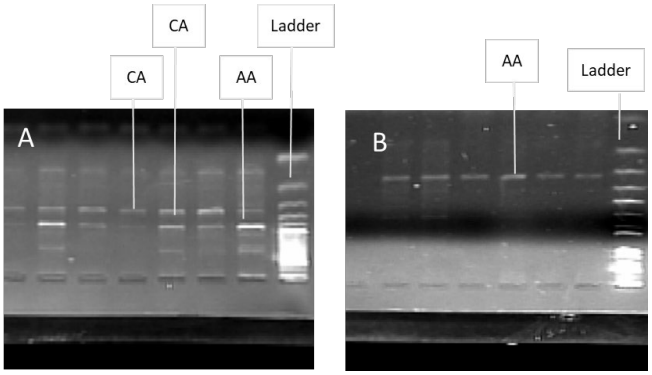

Figure 1. Results of ITPA genotyping. (A) rs1127354 (B) rs7270101

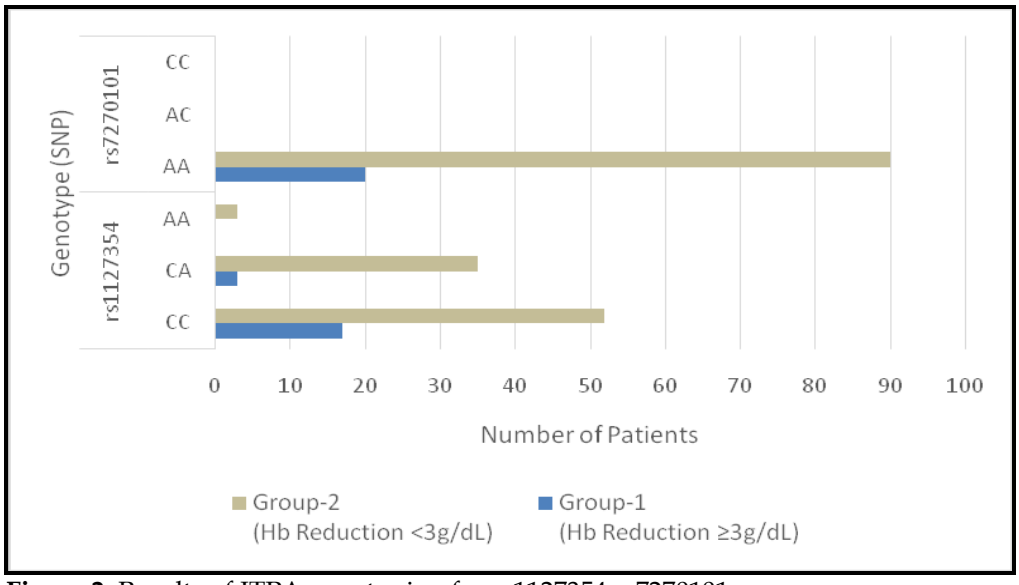

Figure 2. Results of ITPA genotyping for rs1127354 rs7270101

At rs1127354, 69 patients showed the presence of CC genotype, 38 were found to have CA and 3 patients had AA genotype. Out of the 69 CC patients 17 patients were anemic while 52 patients were non-anemic. 3 out of 38 CA patients were anemic while 35 were non-anemic. All the 3 AA patients at rs1127354 were non-anemic. Results of ITPA genotyping at rs1127354 are shown in Table 3.

The minor allele A at rs1127354 was found to be protective against anemia due to interferon and ribavirin combination therapy in hepatitis C patients (Odds ratio=0.275, C.I= [0.081-0.938], Chi2=4.77, p=0.02889) while the major allele C at rs1127354 was found to be associated with anemia in HCV patients receiving interferon and ribavirin combination therapy (Odds ratio=3.638, C.I= [1.066-12.409],
Chi2=4.77, p=0.02889).
At rs7270101 all the patients showed AA genotype. Due to the absence of non-AA genotypes at rs7270101, we were unable to calculate the effect size of CA and CC genotypes.

Discussion

Our study shows an association between anemia due to peginterferon plus ribavirin combination therapy and ITPA polymorphisms. Patients with CA/AA genotype at rs1127354 had less Hb reduction after 3 months of interferon plus ribavirin treatment with respect to CC genotype. This finding is similar to the studies conducted in western countries. Thompson *et al* (14) included 238 HCV patients in their study and found that ITPA genotypes at rs1127354 were 215 CC, 20 CA, and 3 AA. Similarly at rs7270101 ITPA genotypes were 188 AA, 46 AC and 4 CC. They concluded that ITPA polymorphisms rs1127354 & rs7270101 are associated with anemia due to RBV & Peg INF therapy such that minor alleles of both these polymorphisms provide protection against anemia.
The results of our study show that at rs7270101 all the patients were AA genotype and none CA/CC genotype. This finding is similar to the study of Kim *et al* (15) who included 133 Korean patients in their study and found that 108 of these patients were CC and 25 were non- CC at rs1127354 (groups A & B respectively). On the other hand at rs7270101 all 133 patients were AA. They concluded that ITPA non CC genotypes at rs1127354 are associated with less decrease in hemoglobin level due to RBV & Peg INF combination therapy in HCV patients.

The study is delimited by the smaller number of study patients. Absence of CA/CC genotypes at rs7270101 in our study may be attributed to this limitation. Studies exploring the effect in larger group of patients are, hence, suggested.

Conclusion

The current study concludes that CA/AA genotype at ITPA SNP RS1127354 provides protection against anemia in hepatitis C patients receiving interferon and ribavirin combination therapy.

Conflict of Interest: Authors declare no conflict of interest.

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Authors' Contribution

IUR: study conduction, data analysis and manuscript writing

SS: Study conception, study conduction and data analysis

MA: Study conduction

MTMK: Data analysis and manuscript writing