The Relation between Inosine Triphosphatase Gene Variants and Anemia and Thrombocytopenia Induced by Combined Pegylated Interferon-Alfa and Ribavirin Therapy among Chronic Hepatitis C Patients in Egypt

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The goal of such a treatment is to reach the Sustained Virological Response (SVR), defined as undetectable HCV RNA in the serum after 24 weeks of post-treatment follow-up. The achievement of SVR after therapy can prevent liver-related complications and improves survival [3]. However, less than 50% of patients infected with HCV genotype 4 achieve SVR or are cured of the infection following this type of therapy [4]. A number of host and viral factors have been linked to the response to therapy [5]. Recent studies have revealed a genetic polymorphism in the region of the interleukin 28B gene (IL28B) encoding IFN-lambda that is strongly associated with viral clearance following PEG-IFN-α/RBV therapy [6].

In addition, treatment is often poorly tolerated due to side effects, particularly RBV-induced hemolytic anemia [7]. Some patients are thus prevented from completing therapy or require RBV dose reduction that increases the risk of treatment failure [9]. Variation in the degree of RBV-induced hemolysis and anemia between individuals is likely affected by both clinical and genetic factors. Genetic variation of inosine triphosphatase (ITPA) causing an accumulation of inosine triphosphate (ITP) has been shown to protect patients against ribavirin (RBV)-induced anemia during treatment for chronic hepatitis C infection by genome-wide association study (GWAS). Inosine triphosphate pyrophosphatase confers protection against RBV-induced ATP reduction by substituting for erythrocyte GTP, which is depleted by RBV, in the biosynthesis of ATP. Because patients with excess ITP appear largely protected against anemia, these
results confirm that RBV-induced anemia is due primarily to the effect of the drug on GTP and consequently ATP levels in erythrocytes [10]. ITPA SNP, rs1127354, genotypes AA and CA are significantly associated with less absolute reduction in Hb levels, especially during the early weeks of therapy, and protect against the development of severe anemia. Interestingly, the CC genotype had significantly less reduction in the mean platelet count compared with the AA/CA genotype due to a reactive increase of platelet count through weeks 1-4. So, rs1127354 in the ITPA gene was independently associated with RBV-induced severe anemia and IFN-induced thrombocytopenia [11]. In addition to RBV-related hemolytic anemia, bone marrow suppression is an important adverse effect of PEG-IFN therapy. The resulting neutropenia and thrombocytopenia leads to a decrease in medication dose or premature withdrawal from therapy in 10% to 14% of patients [12]. The decline in platelet counts that occurs during antiviral therapy is less pronounced when IFN is combined with RBV than when administered alone, suggesting RBV may also play a role in thrombocytopenia [13]. A genome-wide association study in Japanese HCV patients found that the ITPA SNP (rs1127354) CA/AA genotype was significantly associated with a lower absolute decrease in Hb levels, especially during the early weeks of therapy, but independently associated with a greater decrease in platelet counts [14]. ITPA gene variants that protect against anemia have been established among Caucasian, Hispanic, African-American, and Japanese populations [15,16].

Material and Methods

In this prospective study, 149 Egyptian chronic hepatitis C patients (positive for HCV RNA for more than 6 months) treated with PEG-IFN-α/RBV therapy were enrolled from May 2012 to December 2012. At Ismailia Center for Treatment of viral Hepatitis-located in Ismailia Fever Hospital-in Egypt and informed consent was obtained for the analysis of peripheral blood for DNA extraction and genetic testing. All patients were eligible for treatment with combined therapy according to inclusion and exclusion criteria of Egyptian National committee for control of Viral Hepatitis. All participants were treatment-naïve patients with no evidence of any associated liver diseases other than chronic viral hepatitis C. All subjects gave written informed consent to participate in the study according to the process approved by the Ethics Committee of Faculty of medicine Suez Canal University. Patients received weekly injections of PEG-IFN-α-2b (1.5µg/kg body weight) or PEG-IFN-α-2a (180µg) plus oral administration of RBV (800 to 1,200mg daily depending on body weight). On-treatment dose reduction and discontinuation of PEG-IFN-α or RBV were chosen based on the recommendations stated in package inserts or clinical circumstances of individual patients to avoid possible side-effects. All patients were subjected to history taking, physical examination, laboratory investigations, liver biopsy and ITPA gene variant (rs1127354) analysis by Taqman Real Time PCR. Follow-up was done at weeks 1, 2, 3, 4, 8, and 12 weeks for symptoms and laboratory evidence of adverse reactions caused by combined therapy.

DNA extraction and TaqMan SNP assay:

Genomic DNA was extracted from the whole blood of each patient using a genomic DNA extraction kit (QIAGEN®kit). Genetic polymorphisms (ITPA SNPs) located in exon 2 (rs1127354) of the ITPA gene on chromosome 20 determined using real-time detection polymerase chain reaction (Step One, real time PCR machine, Taqman allelic discrimination kit) for 149 treatment-naïve Egyptian patients with chronic HCV. In the present study, we categorized ITPA, rs1127354, CC as the major variant and CA/AA as minor variants.

Statistical analysis:

Statistical analyses were conducted using Statistical Package of Social Sciences version 14 (SPSS version 14 Inc., Chicago, IL, USA). Categorical variables were presented as frequencies and percentages while continuous variables were reported as the means ± SD (range). According to the type of data, the following tests were used to test differences for significance; Chi square and Student t-test with least significance difference. The statistical analysis of comparisons of the categorical variables of the groups was performed by Chi Squares and Fischer's exact tests, Student t-test was used to compare the direct continuous variables between groups with a significant p-value of less than 0.05. The assessment of correlation coefficient, Odd's ratio and multiple logistic regression analysis were used to determine relationships between different dependent and independent variables and the nature of these relationships. Allele and genotype frequencies were evaluated for their association with cytopenia using Fisher's exact tests.

Results

This study was conducted as a prospective study. The study was performed on the data collected from 149 patients with chronic HCV infection who were eligible for standard combined
PEG-IFN/RBV therapy in the center for treatment of viral hepatitis in Ismailia Fever Hospital.

1- Patient characteristics:

The clinical characteristics and SNP genotypes of the 149 patients included in our study are summarized in (Table 1); they had a mean age of 43.4 year, a mean BMI of 26.6 and disease duration of 26 months. Their mean stage of fibrosis and grade of activity 1.9 and 2 respectively. The results also shows that 137 (91.9%) had CC genotypes of ITPA rs1127354 and 12 (8.1%) had CA genotype.

Comparing baseline characteristics of patients with CC genotype of rs1127354 compared to CA (p=0.009) (Table 2).

Comparing mean values of hemoglobin reduction was significantly higher in subjects with CC genotype of rs1127354 compared to CA at weeks 1, 2, 4, 8, and 12 (p<0.0001 for each) (Table 3).

Comparing HB decline by >3gm/dl at week 4 compared to non-CC (p=0.002) (Table 6).

During the 12 weeks of therapy the frequency of anemia was significantly higher in CC patients compared to CA patients (65.7% vs. 16.7%) (p=0.002). CA genotype of ITPA is a protective variant. Mean while the prevalence of this genotype is low in the studied subjects, (Table 5).

Table 3: Means values of Hb reduction during the first 12 weeks of therapy according to ITPA rs1127354 genotypes.

Comparing HB decline by >3gm/dl up to week 12 according to ITPA genotypes.

Table 5: Frequency of patients with HB decline >3 gram/dl.

Reduction of platelet counts by more than 30,000/µl was significantly more frequent in patients with CA compared to CC (75% vs. 29.2%, p=0.002) (Table 6).

Table 6: Frequency of platelets reduction >30,000/mm³ at week 4 according to ITPA rs1127354 genotypes.
During the first 12 weeks, RBV dose reduction was recommended in 75 patients, 73 with CC and 2 with CA (16.2% vs. 53.3%, p=0.017), (Table 7).

Table (7): Frequency of Ribavirin dose reduction according to ITPA rs1127354 genotypes.

<table>
<thead>
<tr>
<th>RBV dose reduction</th>
<th>CA</th>
<th>CC</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>2 (16.7%)</td>
<td>73 (53.3%)</td>
<td>0.017*</td>
</tr>
<tr>
<td>No</td>
<td>10 (83.3%)</td>
<td>64 (46.7%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>137</td>
<td></td>
</tr>
</tbody>
</table>

By logistic regression analysis we found that female sex (p<0.0001), ITPA genotype CC (p<0.0001), Baseline Hb <15gm/dl (p<0.0001) are independent predictors for occurrence of anemia during the first 12 weeks of therapy (Table 8).

By regression analysis we found that female sex (p=0.0008), ITPA genotype CA (p=0.001), Baseline Plt. (<200 X 10^3) (p=0.02) and baseline Hb (<15) (p=0.01) are independent predictors for occurrence of thrombocytopenia during the first 12 weeks of therapy (Table 9).

### Discussion

This study was designed to study the relation between ITPA genotypes and incidence of cytopenias in chronic hepatitis C patients treated with pegylated interferon plus ribavirin. In this study, the median age of patients was 45 years, with a range of 19 to 59 years. Meanwhile, the median duration of liver disease since diagnosis of chronic HCV infection was short (12 months) and the median BMI was 26.6Kg/m^2. Furthermore, the median values and ranges of liver fibrosis stage and grade of activity denoted mild to moderate liver disease associated with HCV. It aims to achieve better sustained virological response in view of the limited financial resources provided by the Ministry of Health. The mean age of our patients was 43.4 years, being higher than reported in an Egyptian series [17] and in similar series in Europe and USA [18,19]. The difference in age could be related to the presence of an active surveillance system that could discover infected individuals at an earlier age. In Egypt, there is currently no ongoing surveillance of chronic HBV and HCV, with the exception of monitoring of infection rates in hemodialysis units [20]. The study subjects were 89 males and 60 females with a male to female ratio of 1.48:1; being lower than 1.65:1 and 2.35:1 reported in two similar studies done earlier in the same center [21,22]. While this male predominance reflects the higher infection rate of males in the community [23]. It can also be explained by the...
frequent checkup of males in many settings as before employment, military service or travelling abroad. Moreover, the difference could be related to sex difference in severity of liver disease due to chronic HCV infection. In this study mean BMI of patients was (26.6±2) that nearly equal to another Egyptian study patients group (25.6±2.7) [17] and was high in comparison to Japanese patients (22.3±2.8) [24].

Distribution of ITPA gene variants among studied population:

In this study, the frequency of the ITPA rs1127354 genotypes CC and CA was 92% and 8% respectively of 149 patients with chronic hepatitis C. None had AA genotype. The frequency of CC genotype is comparable with recent reports among the Egyptian population [17,25]. However, Egyptian distribution of CC is higher than that reported in Taiwan (72.9%), Japan (75.1%) Korea (81.2%) and Spain (80.8%) [26-29]. The minor allele distribution of ITPA polymorphism in this study was 4%, which is similar to that described by Ahmed, et al. [17] and close to 5-7% found in Caucasians and Africans. The highest frequency of the minor allele A was described among Asians (11-19%) and lowest among Central and South Americans (10-2%) [30,31].

Hb decline and ITPA genotype:

The decline of HB >3gm at week 4 was noticed in 55 (36.9%) patients and reduction of platelets >30,000/dl was found in 49 (32.9%). Severe anemia (<10gm/dl) necessitating reduction of ribavirin dose occurred in 75 (50.3%) during therapy. The frequency of anemia in this study is higher than 54% [32].

In this study, although Hb levels decreased with therapy in all patients, a high rate of decline was noticed following treatment till week 4 and the decline continued at a slower rate thereafter till week 12.

The association of Hb decline with ITPA rs1127354 genotype CC was evident all through the study duration up to week 12. These findings were similar to that reported by Ahmed, et al. 2013 who reported significantly lower mean values of Hb levels in CC patients continued till week 30 compared to non-CC. Compared to our results, in Korea, Kim and colleagues reported a lower percentage of anemia (50%) among CC patients and a similar percentage (16%) in non-CC patients [28]. However, the lower percentage of CC genotype in their series (81.2%) could explain the lower prevalence of anemia compared to the present study (67%).

Ribavirin dose reduction, anemia and ITPA genotype:

Although anemia was present in 122 patients during therapy, marked anemia (Hb <10gm/dl) necessitating reduction of ribavirin dose was reported in 75 (50.3%) patients. The frequency of dose reduction was affected by the dose of ribavirin and genotype of ITPA rs1127354. A daily ribavirin dose of ≥1200mg was significantly associated with higher frequency of dose reduction compared to patients given 1000 or 800mg daily (63.2% vs. 19.5%). The high initial dose of ribavirin had an odds ratio of ribavirin dose reduction of 7.5 (p<0.0001, 95% CL 3.17-17.8). Similarly, patients with CC genotype compared to non CC genotype showed significantly higher frequency of ribavirin dose reduction (53.5% in CC vs. 16.7% in non CC, p=0.0170. The odd ratio of CC patients to reduce the dose of ribavirin was 5.7 (p=0.028, odds ratio=5.7, 95% CL (1.2-27.0)). The frequency of ribavirin dose reduction was lower than that reported among Egyptian patients (77.4% vs. 44.4%, p=0.044) and higher than that in a Taiwanese series (42% and 11.8% of CC and non-CC Chinese patients) [17,26]. The high frequency of dose reduction as a management of severe anemia reflects the high rate of anemia in view of the low rate of protective non CC genotype of ITPA rs1127354 in our series. Furthermore, despite the importance of adding ribavirin to pegylated interferon to increase the virological response, reduction of its dose is an acceptable solution to decrease the degree of anemia and maintain therapy. Alternatively, stopping of ribavirin or adding Erythropoietin could be practical solutions in patients with marked anemia. Recombinant erythropoietin (EPO) has a beneficial role of in alleviating ribavirin-induced anemia, improving quality of life, enabling higher ribavirin dosage and consequently improving SVR. However, no general consensus exists regarding the use of EPO for specific indications: Its optimal dosing, treatment benefits and potential risks or cost efficiency [28].

In this study recombinant EPO was not an affordable alternative due to its high cost. The frequency of ribavirin dose reduction in this study was higher than 40.1% reported in treated Korean patients with chronic hepatitis C genotype 1 [34].

In You's study, anemia was reported in 60.8% of patients on high ribavirin dose (≥15mg/kg body weight) and 29.26% with low doses. In this setting, the distribution of CC genotype of ITPA rs1127354 in Korean patients was lower than reported by the current study (81.2% vs. 92%) [28].
Despite the side effects reported in patients treated with ribavirin, it is a valuable drug that increase the virological response in pegylated interferon based regimen. More recently, the importance of ribavirin has been demonstrated in a clinical trial where interferon was employed with telaprevir. The response rates were lowest in patients who were not administered ribavirin [38].

The impact of ribavirin dose reduction on virological response is controversial. Reduction of ribavirin dose in patients with HB <10gram/dl significantly affect the treatment outcomes [36-38]. In one study, SVR decreased from ~65% to ~45% when ribavirin dosage is reduced from ~15mg/kg to ~7mg/kg of body weight, in combination with pegylated interferon a2b at 1.5µg/kg of body weight [36]. More recently, in a Korean study, you and colleagues reported higher EVR and SVR among patients given ribavirin ≥15mg/kg body weight, compared to lower doses. Interestingly the difference in these two virological responses was not statistically significant despite the higher frequency of ribavirin dose reduction associated with higher ribavirin doses [34]. If the RBV dose is lowered the virologic response may drop, but it was reported that when Peg-IFN was administered sufficiently the virologic response did not drop even if the RBV dose was somewhat lower than the standard [37].

**Platelet reduction and ITPA genotype:**

Before initiation of treatment, the mean platelet count was similar in CC and CA carrying patients. Platelet count decline occurred throughout the 12 weeks of therapy with a marked decrease in platelet count after one week of initiating therapy. Platelet reduction >30,000/dl was significantly more frequent in CA compared to CC patients at week 4 (p=0.002). However, the pattern of platelet decline differed according to genotypes of ITPA rs1127354 along the 12 week of the study. More reduction in platelet count was noticed between week 2 and 8 in CA carrying patients while CC patients showed more reduction after the 8th week. This resulted in a higher mean of platelet decline of CA at week 4 that was not statistically significant (p=0.0833) and a higher mean of platelet decline of CC at week 12 that reached a statistical significance (p=0.0103). This pattern is different from that described by Ahmed and colleagues. They reported significantly higher percentages of decline in platelet counts in non-CC compared to CC throughout the 48 weeks of therapy. The lower platelet reduction among CC patients with marked anemia was also reported by many studies [15,27,39]. Although the association of lower platelet reduction at week 4 with CC genotypes was not significant in this study, it indicates that the anemia-susceptible patients of group CC are less likely to develop a higher degree of platelet reduction than non-CC patients. This pattern was previously explained by the relative reactive increase in platelet count by the active bone marrow in cases with severe anemia [40].

The decline in platelet counts that occurs during antiviral therapy is known to be less pronounced when IFN is combined with RBV than in the setting of IFN monotherapy [13,41]. This has been attributed to a relative thrombocytosis occurring in response to RBV-induced hemolysis [42]. Although many studies confirm this relation between marked anemia induced by ribavirin and less decline in platelet count in CC patients treated by pegylated interferon/ribavirin combination, Jiang and colleagues described a case of severe thrombocytopenia in a CC patient. She was 57 year old female who received pegylated interferon a2a and ribavirin. She showed the non-favorable genotype of ITPA rs1127354 CC and became HCV RNA negative after 9 weeks of therapy. During the therapy, the platelet count remained above 8.0 X 10^4/µl for about 9 months. However, by the 44th week from the beginning of the treatment, a sudden decrease in the platelet count to 0.8 X 10^4/µl was observed. After prednisolone was administered, the platelet count increased. Finally the platelet count had risen above normal range [43]. In this study, both univariate and multivariate analysis showed that female gender, baseline haemoglobin <15gm/dl and CC genotype of ITPA were significantly associated with Hb decline >3gm/dl and CC genotype of ITPA were significantly associated with Hb decline >3gm/dl during the 12-weeks therapy with combined ribavirin/pegylated interferon therapy.

**ITPA and DAADs:**

The value of ITPA SNPs in predicting occurrence of anemia in non-interferon based regimens was recently studied with the direct acting antiviral drugs. In the SOUND-C2 study, Asselah and colleagues studied the side effect of combined faldaprevir, BI 207127 and ribavirin combination for patients with chronic hepatitis C. They reported a low risk of ribavirin induced anemia and ITPA SNPs may be useful as a predictor of haemolysis [44].

Conclusion, functional variants of the ITPA gene, associated with ITTPase deficiency, are protective against RBV-induced hemolytic anemia, reducing the need for RBV dose reduction, and maintaining cumulative RBV dosage, although this study confirmed this association regarding ITPA rs1127354, the low rate of non CC genotype (~8%) present in this work and other two studies from Egypt do not justify testing for ITPA rs1127354
genotypes before starting treatment with ribavirin based regimens. However, it is recommended to assess other variant of ITPA as rs7270 10 1 among a new cohorts of CHC patients.

References


المملوكة العربية

المقدمه والمهدف من البحث: تعد التغييرات السلبية في مكونات الدم التي قد تحدث أثناء العلاج بالإنترفيرون طويل المفعول مع الريبارفيرون في مرض الأنتهاب الكبدى الفيروسي سي المزمن من العوامل الرئيسية التي غالبا ما تتطلب اتخاذ جريمة العلاج وبالتالي انخفاض نسبة نجاحه.

أثبتت الدراسات أن بعض التغييرات من التركيب الجيني لانزيم الإنزيم ثلاثي الفوسفات قد تحمي من فقر الدم وأيضا وجد أنها قد ترتبط بانخفاض أكبر لعدد الصفائح الدموية وذلك أثناء المراحل المبكرة من العلاج المدمج (الإنترفيرون بالإضافة للريبارفيرون) لمرض الأنتهاب الكبدى المزمن سي.

تهدف هذه الدراسة إلى التعرف على العلاقة بين الأشكال الجينية المتعددة لجين إنزيم الإنزيم ثلاثي الفوسفات والأنثى والعناصر المناعية الناتجة عن العلاج المرفوع بالإنترفيرون والريبارفيرون بين مرضى الأنتهاب الكبدى الفيروسي المزمن سي.

طريقة وأسلوب البحث: في هذه الدراسة، التحق 149 من المرضى الساخرين الكبدى المزمن سي من الذين عولموا بعقارى الإنترفيرون مع الريبارفيرون في مركز علاج الفيروسيات الكبدية مستشفى حميات الأسماكية خلال الفترة من مايو 2015 إلى ديسمبر 2016.

تم التحقق من متغيرات التركيب الببتيدات المحددة (SNP) داخل جين إنزيم الإنزيم ثلاثي الفوسفات (ITPA) والرمز (TATAAA127354R). وكذلك تم التحقق من تأثير تلك الانتظارات الجينية لهذا الإنزيم على المتغيرات في المكونات الدموية خلال فترة العلاج.

النتائج: وجد أن المتغيرات الجينية الطفيفة لإنزيم الإنزيم ثلاثي الفوسفات والرمز TATAAA127354R كانت مرتبطة بشكل كبير واحصائي مع الحماية ضد حدوث فقر الدم في الأسبوع الرابع من العلاج وكذلك مرتبطة مع انخفاض أشد بعد الصصفائف الدموية.

الاستنتاجات: إن وجود المتماثل في التركيب الجيني لإنزيم الإنزيم ثلاثي الفوسفات والرمز TATAAA127354R يعد مؤشراً مفيداً لتحديد فقر الدم المسبب بالريبارفيرون وربما تكون ذات صلة بانخفاض أكثر حدة في تعداد الصفائح الدموية في خلال المرحلة الأولى من العلاج المركب لالتهاب الكبدى المزمن سي بين المرضى المصريين.