The Course of Hepatitis C Infection and Response to Anti-viral Therapy in Patients with Thalassemia major and Hepatitis C Infection: A Longitudinal, Prospective Study

Sanaa Kamal¹, Sara Abdelhakam¹, Dalia Ghoraba¹, Mohamed Amer Mohsen², Ahmed Abdel Salam³, Hoda Hassan⁴ and Leila Nabeigh⁵.

¹ Department of Tropical Medicine, Ain Shams Faculty of Medicine, Cairo, Egypt.  
² Department of Radiodiagnosis, Misr University of Science and Technology, Cairo, Egypt.  
³ Department of Pediatrics, Misr University of Science and Technology, Cairo, Egypt.  
⁴ Department of Hematology and Clinical Pathology, Faculty of Medicine, Cairo University, Cairo, Egypt.  
⁵ Department of Pathology, Ain Shams Faculty of Medicine, Cairo, Egypt.

Competing interests: The authors declare no conflict of Interest.

Abstract. **Background:** The course of hepatitis C infection (HCV) in patients with thalassemia has not been adequately studied, and management has not been optimized. The current prospective longitudinal study assessed the clinical course, outcome, progression, and management of recently acquired HCV in patients with transfusion-dependent thalassemia major versus acute HCV without thalassemia.

**Methods:** A well-characterized cohort of patients with thalassemia and recent HCV infection or recent HCV without thalassemia were enrolled and prospectively followed. The blood transfusion needs and chelating agents were determined. Liver functions tests, HCV-RNA, iron, and ferritin levels were measured. Patients with chronic HCV evolution received treatment for HCV. The fibrosis progression rate was determined in chronic HCV patients with or without thalassemia by paired liver biopsies or serial transient elastography (TE), or serum markers of liver fibrosis. Liver iron content (LIC) was assessed by R2 MRI.

**Results:** Self-limited acute HCV was observed in 17% of patients with acute HCV and thalassemia versus 35% of patients without thalassemia (P=0.031). The fibrosis progression rates were significantly higher in patients with chronic HCV and thalassemia compared to those with chronic HCV alone (1.14±0.48) and (0.35±0.14) (P<0.0001), respectively. A direct linear correlation was observed between the fibrosis progression rate and each of LIC (R=+0.67; P=0.01) and ferritin (R=0.77; P<0.01). In patients with chronic HCV and thalassemia, the sustained virologic response (SVR) to pegylated interferon-based therapy and direct antiviral agents (DAAs) were 33% and 82% respectively (P<0.0001), while in chronic HCV patients without thalassemia, the SVR rates to PEG-IFN/RBV and DAAs were 51% and 92% respectively. Five patients with concomitant HCV and thalassemia died during the study due to cardiac causes (n=3) and liver cancer (n=2).

**Conclusions:** Patients with acute HCV and thalassemia have low rates of spontaneous resolution of HCV infection, and the majority develop chronic HCV. Direct-acting antiviral combinations are associated with high SVR rates and low adverse event in treatment naïve and experienced patients with chronic HCV and thalassemia. Liver fibrosis is accelerated in thalassemia patients with chronic HCV; therefore, early diagnosis, treatment with DAAs, adequate iron chelation, and non-invasive monitoring liver status are recommended to prevent cirrhosis and hepatocellular carcinoma.

Keywords: Hepatitis C; Thalassemia; Liver fibrosis progression; Transient elastography; Serum fibrosis markers.
Introduction. Hepatitis C infection (HCV) is a major cause of liver-related morbidity, cirrhosis, hepatocellular carcinoma, and liver transplantation.\(^1\)\(^2\) In Western countries, HCV prevalence ranges between 2% in the United States of America and 1.6% \(^%\) in Europe.\(^3\) Significantly higher incidence and prevalence rates are reported from Southeast Asia, Africa and Western Pacific.\(^1\) In Egypt, HCV has been a huge public health and economic burden with prevalence rates exceeding 15%; however, the incidence of HCV in Egypt is gradually declining after adoption of a nationwide program for prevention and treatment of HCV infection.\(^4\)\(^5\) HCV infection results in acute hepatitis which may be either subclinical or associated with symptoms.\(^6\) The rates of self-limited acute HCV vary; however, more than half of HCV infections progress to chronic hepatitis that may progress to cirrhosis and liver cancer in some patients.\(^7\) Progression of HCV related liver fibrosis is highly variable and may be accelerated in the presence of coinfections such as HIV, HBV, or schistosomiasis or comorbidities.\(^7\)\(^12\)

Beta-thalassemia comprises a group of hereditary disorders characterized by a genetic deficiency in the synthesis of beta-globin chains resulting in chronic hemolytic anemia that requires long-term transfusion therapy and iron chelation.\(^13\)\(^14\) Despite the advances in the management of thalassemia patients, long-term transfusion therapy remains a risk for increased iron compartmentalization in different organs such as the liver, heart, endocrine glands such as pituitary gland, pancreas, ovaries, testes, thyroid, parathyroid and adrenals leading to various complications.\(^13\)\(^16\)

In Egypt, as in several Mediterranean countries, beta-thalassemia represents a major health problem since it constitutes 85% of hereditary hemoglobinopathies. The injurious impact of iron overload on the liver is further accentuated by the high prevalence of HCV infection among patients with thalassemia.\(^17\)\(^18\)\(^19\) Before the adoption of obligatory screening for HCV at blood banks in Egypt, the prevalence of HCV among multitransfused thalassemic patients reached almost 85%.\(^20\)\(^21\) Despite the current strict control on HCV screening in blood banks, a recent study showed that 37% of Egyptians with thalassemia have HCV infection.\(^19\) Given the high prevalence of HCV in Egypt, interfamilial and iatrogenic HCV transmission are important modes of HCV transmission among thalassemics in Egypt.\(^21\) Concomitant HCV infection and iron overload are major causes of advanced liver fibrosis and cirrhosis among Egyptian multi-transfused thalassemia patients.\(^22\)\(^23\)

Accurate assessment of the liver fibrosis progression rates requires performing at least one baseline liver biopsy for the initial diagnosis and staging of liver fibrosis, and successive (one or more) liver biopsies performed several years after the baseline biopsy. However, liver biopsy is an invasive procedure that may be associated with complications and may not be ethically justified if patients are not offered therapy. Histopathologic scoring systems are limited by biopsies' size, variability of inter- and intra-observer reproducibility, sampling errors, and potential fibrosis heterogeneity throughout the liver.\(^24\) Thus, non-invasive methods for assessment of HCV related hepatic fibrosis including direct or indirect serum biomarkers of fibrosis or assessment of liver stiffness by transient elastography or magnetic resonance elastography have been used to determine the degree of liver disease and predict the rate of hepatic fibrosis progression in patients with chronic HCV to prioritize therapy to those with accelerated rates of hepatic fibrosis progression.\(^25\)\(^28\)

To date, neither the course of HCV infection in patients with thalassemia nor their response to antiviral therapy particularly direct-acting antiviral agents (DAAs) has been adequately assessed in longitudinal studies. Therefore, we conducted this prospective, longitudinal study to investigate the clinicopathologic features, course, progression of disease in patients with thalassemia and recent HCV infection. We also evaluated the diagnostic and prognostic performance of transient elastography and various panels of non-invasive fibrosis biomarkers individually or in combination to assess their potential role as non-invasive diagnostic tools for monitoring liver fibrosis in patients with thalassemia who developed chronic hepatitis C.

Patients and Methods.

Study design and study population. We conducted the current longitudinal, prospective study at six hospitals and liver centers in Cairo, Upper Egypt and Delta Egypt, between February 2004 and December 2018.
Consecutive Egyptian thalassemia patients with proven acute HCV genotype 4 (HCV-G4) infection were enrolled in the current study in addition to patients with proven acute HCV mono-infection. The study was approved by the Office for Human Protections Research Board of the participating institutions. The protocol and all procedures of the study were conducted in accordance with Good Clinical Practice guidelines and in conformity with the ethical guidelines of the Declaration of Helsinki. All patients presented written informed consent before enrollment and before any study-related procedure.

Patients with thalassemia and recent HCV infection were invited to join the study if they fulfilled the criteria of acute HCV which include elevated serum alanine aminotransferase (ALT) at least five times the upper limit of normal (40 U/L), seroconversion from a previous documented negative HCV antibody test prior potential HCV exposure into a positive antibody test after a suspected risky exposure (anti-HCV tested using a Cobas e411 analyzer with Elecsys Anti-HCV assay; Roche Diagnostics, Mannheim, Germany) and recent detection of HCV (PCR; COBAS Amplicor HCV test version 2.0; Roche Molecular Systems, Pleasanton, CA, USA) in the presence or absence of symptoms such as jaundice, dark urine, malaise, abdominal pain.

Patients were excluded from the study if he/she had previous HCV infection, hepatitis A, hepatitis B, autoimmune hepatitis, alcoholic liver disease, drug-induced hepatitis, and decompensated liver disease with a history of variceal hemorrhage, ascites, or hepatic encephalopathy, clinical symptoms of cardiac dysfunction; coinfection with schistosomiasis or human immunodeficiency virus (HIV), a leucocyte count less than 3000/mm$^3$, neutropenia (<1500 cells/mm$^3$), serum creatinine above the upper limit of normal (ULN); significant proteinuria (urinary protein/creatinine >1.0 mg/mg), thrombocytopenia (<90 000 cells/mm$^3$), or organ transplantation.

Enrolled patients were followed for spontaneous resolution of acute HCV or development of chronic HCV. Spontaneous HCV clearance was defined as undetectable HCV RNA tested at least in two instances six months after the estimated seroconversion date or the first positive HCV PCR or date of potential exposure. Detectable viremia beyond six months implies chronic hepatitis C. Patients with proven chronic HCV were screened for eligibility to treatment and followed during therapy and after treatment completion (Figure 1). Patients who were ineligible to therapy or those who declined therapy were enrolled and followed for assessment of hepatic fibrosis progression or consideration for DAAs. As control groups, patients with chronic HCV without thalassemia were also enrolled and followed for at least five years.

**Laboratory assays.** Complete blood picture, liver functions tests (aspartate aminotransferase (AST); alanine aminotransferase (ALT); alkaline phosphatase (ALP) and gamma-glutamyltransferase (GGT); conjugated and unconjugated bilirubin levels) were performed monthly for all patients at enrollment and during the acute phase and every three months in the chronic phase of HCV infection. Serum iron, ferritin (electrochemiluminescent immunoassay, ELECSYS 2010; Hitachi High Technologies Corp, Tokyo, Japan; Ferritin values exceeding 500 ng/mL) indicated iron overload, serum iron (mg/dl).

- **Qualitative HCV-PCR (COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test, v2; Roche Diagnostics Branchburg, NJ, USA)** with low limit of detection (LoD) = 15 IU/mL was performed to at all patients after potential risky exposure then repeated after 4 weeks to monitor the first positive HCV positivity. Quantitative HCV-PCR (Lower limit of quantitation (LLoQ): 15 IU/mL (OBAS® AmpliPrep/COBAS® TaqMan® HCV Test), was performed at weeks 12, 24 and 48 weeks to all patients. In patients with chronic HCV evolution eligible for HCV therapy, quantitative HCV-PCR was conducted before initiation of treatment, at treatment weeks 4, 12, 24, end of therapy and 12 or 24 weeks after therapy completion according to the treatment regimen.

---

**Interventions.** **Antiviral therapy for chronic HCV:** Until 2014, patients with chronic HCV were treated with 48 weeks of PEG-IFN α-2a (180 μg, Pegasys; Hoffman La Roche, Basel, Switzerland) and ribavirin (RBV) (10.6 mg/kg/day; Copegus, Hoffman La Roche, Basel, Switzerland) for patients without thalassemia or RBV 600 mg/day in patients with thalassemia. Beyond 2014, patients received with sofosbuvir 400 mg and daclatasvir 60 mg daily for 12 weeks. The primary endpoint was sustained virological response (SVR) defined as undetectable serum HCV RNA 12/24 weeks after the discontinuation of treatment (according to the therapeutic regimen) using COBAS® AmpliPrep/COBAS® TaqMan® HCV Quantitative Test, v2.0 (Roche Diagnostics, Branchburg, NJ, USA) with a lower limit of detection (LoD) of 15 IU/mL.

**Iron chelation:** The history of iron chelation, age of start of chelation therapy, frequency of chelation and type of chelators were reported in all enrolled thalassemia patients. Patients received deferoxamine (DFO) or deferasirox (DFX) or a combination of both.

**Histological Assessment.** After obtaining each patient’s consent, patients who developed chronic HCV in both groups were subjected to a liver biopsy as a requirement for assessing their eligibility to therapy. A subset of patients who developed chronic HCV and either failed or did not receive PEG-IFN/RBV therapy had second liver biopsies during screening for DAA treatments.
Figure 1. Flow of patients through the study.

treatment. Liver biopsies were performed under ultrasound guidance with local analgesia. Biopsies were divided into two sections: one for histology examination and another for liver iron concentration (LIC). Biopsies were stained by hematoxylin-eosin, Masson’s trichrome, and Perl’s stains and evaluated histologically by an experienced pathologist (L.N.). Perl’s stained slides were assessed for liver iron according to Scheuer and Rowe in which stainable iron is graded on a 0-4 scale where 0 implies absence of granules at magnification x 400, grade 1 indicates granules are barely discernable at x 250 magnification and easily confirmed at x 100, grade 2 is considered when discrete granules were resolved at x 100 magnification, grade 3 depicts discrete granules resolved at x 25 magnification while grade 4 is given when masses are visible at magnification x 10 or naked eye. LIC was determined by atomic absorption spectrophotometry with normal LIC levels ranging between 0.4 and 2.2 mg/g of dry liver weight. (dw). LIC values of between 3 and 7 mg/g dw are considered mildly elevated while values between 7-14 are moderate and LIC >14 mg/g dw represent severe iron overload.

Liver biopsies were graded according to the Metavir scoring system. Briefly, the METAVIR scoring
system assesses histologic lesions in chronic hepatitis C using two separate scores, one for the necroinflammatory grade (A for activity) and another for the stage of fibrosis (F). These scores are defined as follows: stages of fibrosis (F): F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with rare septa, F3, numerous septa without cirrhosis; F4, cirrhosis. Grade for activity (A): A0, no histologic necroinflammatory activity; A1, minimal activity, A2, moderate activity, A3, severe activity.

Fibrosis Progression Rate Assessment. In chronic HCV patient with paired liver biopsies, the direct progression rate of fibrosis per year was estimated as the difference between fibrosis scores of the baseline and follow-up biopsies divided by the interval between the two biopsies. In patients with a single biopsy, the indirect fibrosis progression rate per year was estimated as the ratio between the fibrosis stage (in METAVIR score) and the estimated duration of infection in years.33,34

Transient Elastography. Transient elastography (TE) was annually performed in patients with chronic HCV with or without thalassemia to monitor liver stiffness (LSM) and fibrosis progression by using Fibroscan® (Echosens, Paris, France) device according to the manufacturer’s instructions and as previously described. The liver stiffness measurement (LSM) results were reported in kilopascals (kPa) where higher kPa reflected a stiffer liver and more severe liver fibrosis. According to the TE values, patients are grouped into three categories: those with elastography values of \( \leq 7.0 \) kPa corresponding to METAVIR stages F0 or F1; those with elastography values \( > 7 \) kPa \( \leq 15 \) kPa who have moderate to severe fibrosis (stages F2 and F3) and are at risk for fibrosis progression and the third group includes patients with high elastography values \( > 15.0 \) kPa (METAVIR stage of F4 or some cases of F3) who have a high likelihood of cirrhosis.35,36

Serum fibrosis biomarkers. In patients with chronic HCV with or without thalassemia, serial measurements of the fibrosis markers: human N-terminal procollagen III propeptide (PIINP) (BioSource -International Inc. Nivelles, Belgium), YKL-40 (YKL-40 ELISA Kit, LifeSpan Biosciences Inc. Seattle WA, USA) and serum hyaluronic acid (HA, Hyaluronic Acid Test Kit (Corgenix, Westminster, Colorado, USA) was performed according to the manufacturers’ instructions.

Liver Iron Content assessment by R2 MRI method. Liver iron content was assessed non-invasively in a subset of patients by FerriScan® R2-MRI using a 1.5T scanner (MAGNETOM Avanto Fit, Siemens Healthcare, Erlangen, Germany) as previously described.29,30 LIC values were expressed in mg Fe/g dry weight (dw). According to the FerriScan values, LIC levels were graded as: Grade 1=normal LIC < 3 mg Fe/g dw, Grade 2=mild overload LIC 3–7 mg Fe/g dw, Grade 3=moderate LIC overload 7–15 mg Fe/g dw, and Grade 4=severe LIC overload ≥15 mg Fe/g dw.37,38

Statistical analysis. Continuous variables were expressed as mean ± SD or median (range). Continuous variables that follow a Gaussian distribution were analyzed using unpaired t-test (two unpaired groups) or one-way ANOVA (three or more unmatched groups) or repeated measures ANOVA (three or more matched groups). Nonparametric tests, Mann-Whitney test, and Kruskal-Wallis tests were applied for the analysis of variables that followed non-Gaussian distribution. Categorical variables were compared by chi-square test or Fisher’s exact test. Association between two variables was performed by Pearson or Spearman correlation according to the type of data. Survival time/time to event was measured by Kaplan Meier survival curve or Cox proportional hazard regression. Formal hypotheses were two-sided with a type I nominal error rate of 0.05. Results were expressed as mean values ± standard deviation (SD). For all statistical purposes, \( P < 0.05 \) was considered statistically significant. All statistical analyses were performed using SPSS (Statistical Package for Social Sciences) software version 22 (IBM, Armonk, New York, USA).

Results. From 2004 through 2018, 57 patients with β-thalassemia and recent HCV infection (Group A), and 69 patients with acute HCV without thalassemia (Group B) fulfilled the inclusion criteria, provided informed and were enrolled in the study (Figure 1). Baseline demographic and clinical characteristics of enrolled patients are shown in Table 1. No significant differences in age, gender, or BMI. The risk factors for HCV transmission were comparable between the two groups except for blood transfusion. Patients with concomitant HCV and thalassemia showed significantly reduced hemoglobin levels and total iron-binding capacity, as well as elevated serum iron, transferrin, and ferritin levels in comparison to those with acute HCV infection without thalassemia (Table 1). During the acute phase of HCV infection, the mean total ALT and AST levels and HCV-RNA levels were slightly higher in patients with HCV and thalassemia compared to those without thalassemia although the difference was not statistically significant. (Figure 2).

Outcome of acute HCV in patients with thalassemia and without thalassemia. Spontaneous resolution of acute HCV occurred in 10 out of 57 patients (17.54%) with concomitant acute HCV and thalassemia compared to 24 out of 69 patients without thalassemia (34.78%) (P= 0.043) (Figure 1). Other than the abnormal baseline iron profile, no significant
Table 1. Baseline demographics, clinical characteristics and laboratory results of enrolled patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Concomitant acute HCV and β-thalassemia (Group A) N=57</th>
<th>Acute HCV without thalassemia (Group B) N=69</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>28.52±9.26</td>
<td>32.21±12.48</td>
<td>0.07</td>
</tr>
<tr>
<td>Male: Female</td>
<td></td>
<td></td>
<td>0.44</td>
</tr>
<tr>
<td>Body mass index (BMI) kg/m$^2$ (Mean ± SD)</td>
<td>23.27±3.5</td>
<td>29.39±4.2</td>
<td>&lt;0.0001* **</td>
</tr>
<tr>
<td>Splenectomy; n (%)</td>
<td></td>
<td></td>
<td>0.0001 **</td>
</tr>
<tr>
<td>Risk factors for HCV transmission (n,%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Blood transfusion</td>
<td>2 (3.5)</td>
<td>0 (0)</td>
<td>0.22</td>
</tr>
<tr>
<td>- IV drug use</td>
<td>5 (8.8)</td>
<td>7 (10.2)</td>
<td>1.000</td>
</tr>
<tr>
<td>- Therapeutic injections outside health facilities</td>
<td>10 (17.5)</td>
<td>11 (15.9)</td>
<td>0.81</td>
</tr>
<tr>
<td>- History of recent dental procedures</td>
<td>8 (14)</td>
<td>12 (17.4)</td>
<td>0.63</td>
</tr>
<tr>
<td>- Needle stick or sharp injuries</td>
<td>9 (15.8)</td>
<td>10 (14.5)</td>
<td>1.000</td>
</tr>
<tr>
<td>- Invasive procedures performed by non-medical personnel including home deliveries and wound suturing</td>
<td>6 (10.5)</td>
<td>10 (14.5)</td>
<td>0.59</td>
</tr>
<tr>
<td>- Interfamilial</td>
<td>9 (15.8)</td>
<td>8 (11.6)</td>
<td>0.60</td>
</tr>
<tr>
<td>- Sexual</td>
<td>1 (1.8)</td>
<td>3 (4.3)</td>
<td>0.63</td>
</tr>
<tr>
<td>- Unidentified</td>
<td>7 (12.3)</td>
<td>8 (11.6)</td>
<td>1.0</td>
</tr>
<tr>
<td>Symptoms (jaundice, abdominal pain, fatigue)</td>
<td>19 (33.33)</td>
<td>27 (39.13)</td>
<td>0.0024*</td>
</tr>
<tr>
<td>Years on transfusion/chelation therapy</td>
<td>16.2 ± 5.1</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Frequency of transfusion per year (Mean ± SD)</td>
<td>8.73 ± 4.37</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Chelation therapy, n (%)</td>
<td>5 (8.77)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>- No chelation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Deferoxamine (DFO) monotherapy</td>
<td>20 (48.7)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>- Deferasirox (DFX) monotherapy</td>
<td>20 (34.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Combined DFX and DFO</td>
<td>12 (16.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dL); mean ±SD</td>
<td>9.1 ± 2.1</td>
<td>12.3±2.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total leucocyte count (×10$^9$/L)</td>
<td>6.83±4.36</td>
<td>8.13±3.48</td>
<td>0.0747</td>
</tr>
<tr>
<td>Platelets (per microliter of blood)</td>
<td>182,600±38,900</td>
<td>185,000±47,385</td>
<td>0.7712</td>
</tr>
<tr>
<td>Serum Iron, µg/dL (mean ±SD) (normal range 60–180 µg/dL)</td>
<td>303.25±78.65</td>
<td>88.17±19.64</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ferritin mean ±SD (ng/dL); (range)</td>
<td>2987.14±1572.53</td>
<td>158.81±64.86</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean total bilirubin ± SD (mg/dL)</td>
<td>4.92±6.9</td>
<td>6.02±4.24</td>
<td>0.13</td>
</tr>
<tr>
<td>Mean ALT ± SD (U/liter)</td>
<td>809.83±36.51</td>
<td>796.45±48.17</td>
<td>0.1119</td>
</tr>
<tr>
<td>Mean AST ± SD (U/liter)</td>
<td>758.94±29.3</td>
<td>754.1±55.26</td>
<td>0.6429</td>
</tr>
<tr>
<td>HCV-RNA (x10$^5$/IU/ml)</td>
<td>1.320±0.35</td>
<td>0.834±0.590</td>
<td>0.003*</td>
</tr>
<tr>
<td>HCV genotypes HCV-genotype 4; n (%)</td>
<td>57 (100)</td>
<td>69 (100)</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Values are N (%) or mean ± SD. *P-values from Fisher’s exact test for categorical variables and Wilcoxon rank-sum test for continuous variables. **Significant, ***Highly Significant.

differences in demographics or clinical features were observed between patients who achieved spontaneous HCV clearance compared to those who did not (data not shown).

Response rates to the antiviral treatment regimen in patients who developed chronic HCV with and without thalassemia. Through the study, 47 and 45 patients in groups A and B respectively developed chronic HCV and were assessed for eligibility to either PEG-IFN based therapy (before 2014) or DAAs (after 2014) according to the standard of care available. In Group A, 39 patients were eligible to pegylated interferon and ribavirin therapy, and 8 treatment naïve patients enrolled after 2014 were treated with DAAs. In group B, 47 patients developed chronic HCV; of them, 42 patients received treatment (23 IFN-based therapy and 19 received DAAs) (Figure 1). In both groups, the sustained response rates (SVR) were significantly higher in patients who were treated with DAAs compared with PEG-IFN based therapy. Patients with thalassemia and chronic HCV experienced significantly less 80/80/80 adherence to PEG-IFN with more ribavirin reduction and more adverse events compared...
Figure 2. Kinetics of alanine transferase (ALT) levels and HCV-RNA levels in thalassemia patients with acute HCV (Group A: black line) and patients with acute HCV without thalassemia (Group B: grey line).

to Group B patients. In patients with chronic HCV and thalassemia, the SVR rates were 34.62% and 90% with PEG-IFN based treatment and DAAs therapy respectively (P <0.0001). In patients with chronic HCV without thalassemia, the SVR rates were 60.87% and 94.74% with PEG-IFN/RBV treatment and DAAs therapy respectively (P <0.0001). No significant differences in SVR rates to DAAs were observed between patients with chronic HCV with and without thalassemia (Figure 1).

Blood transfusion demands of patients with HCV and thalassemia during acute HCV infection and during chronic HCV antiviral therapy. During the acute phase of HCV infection, blood transfusion demands were not significantly increased in patients with concomitant thalassemia. However, blood transfusion demands were increased by 15% in patients with thalassemia and chronic HCV during PEG-IFN and RBV therapy, compared with the pre-treatment transfusion amounts. In patients with SVR, the blood transfusion amounts decreased significantly after treatment completion to almost approaching the pre-treatment transfusion demands (6.23 units/month during therapy vs. 5.03 units/month after therapy, p = 0.03) (data not shown).

Liver histology and fibrosis progression rates in the study groups. Liver biopsies were assessed for necroinflammation and fibrosis in patients who developed chronic HCV. Baseline biopsies were performed as a prerequisite for PEG-IFN/RBV eligibility screening (before 2014). The liver biopsies were repeated in treatment-experienced patients who failed PEG-IFN based regimen and were considered for DAAs therapy. The mean interval between the two biopsies was 83.19±18.92 months and 85.53±19.22 months in Groups A and B respectively (P= 0.609).

Baseline and follow-up liver biopsies showed that patients with thalassemia and chronic HCV had significantly higher grading and staging scores (Table 2) The direct liver fibrosis progression rates were assessed in patients with paired liver biopsies (39 and 45 chronic HCV patients with thalassemia and without thalassemia respectively). The fibrosis progression rates were significantly higher in patients with chronic HCV and thalassemia (1.14±0.48) compared to those with chronic HCV alone (0.24±0.14) (P < 0.0001) (Table 2).

Non-invasive assessment of liver fibrosis and fibrosis progression. The liver fibrosis and hepatic fibrosis progression were also monitored non-invasively by serial transient elastography and serum fibrosis markers measurements. At all study time points, TE scores were significantly higher in patients with concomitant chronic HCV and thalassemia compared to Group B patients. The serum markers PIIINP, YKL-40, and HA, were significantly higher in Group A patients compared to Group B patients (Table 3). A significant correlation was observed between histologic liver fibrosis and LSM in Group A patients (r = 0.82 (P< 0.0001)) and group B patients (r=0.69; P<0.001) (Table 4). A correlation was also detected between LSM results and PIIINP, YKL-40 (Table 4).

Liver iron concentration (LIC). At all study points, the liver iron content measured by either histopathology or MRI was significantly higher in thalassemia patients with chronic HCV and thalassemia compared to chronic HCV patients without thalassemia. Heavy hepatic iron overload was observed patients with concomitant HCV and thalassemia during PEG-IFN based therapy (Figure 3a). A significant correlation
occurred between LIC and hepatic fibrosis rates (Figure 3b). Similar results were observed with ferritin (Figures 3 c, 3d). In thalassemia patients with chronic HCV, a linear correlation was detected between LSM values and LIC levels (r= 0.327; P<0.01) while in chronic HCV patients without thalassemia no correlation was found (Table 4).

Follow-up and survival analysis. Fibrosis progression was more accelerated in patients with chronic HCV and thalassemia (Group A) compared to patients with chronic HCV without thalassemia (Group B) (P=0.002; Figure 4a).

Survival analysis revealed a significant difference between the study groups (P=0.04). Five patients with concomitant HCV and thalassemia passed away during the study. The causes of death were cardiac-related in 3 patients and due to hepatocellular carcinoma in two patients. Among patients with chronic HCV without thalassemia, one patient passed away after a traffic accident.

Discussion. The current prospective, longitudinal study compared the outcome and progression of HCV infection in a well-characterized cohort of thalassemia patients who acquired recent HCV infection and a cohort with recent HCV infection without thalassemia. The study also investigated the response to antiviral therapies. The risk factors for HCV infection in this study reflect the previously reported exposures related to acute viral of HCV in Egypt\[4,5,11,12,21\] and highlight the role of interfamilial HCV transmission as previously reported.\[31,39\] In the current study, the rate of spontaneous resolution of acute HCV in patients with concomitant acute HCV and thalassemia was significantly lower compared to the rate of spontaneous eradication of acute HCV mono-infection\[11,12,39,43\] Very few studies investigated the outcome of acute HCV in a limited number of thalassemia patients. A study\[44\] reported spontaneous HCV resolution rate of 44%. It is not clear why patients with thalassemia have lower rates of spontaneous HCV eradication, however, given the limited number of studies, the non-homogeneity of cohorts, the differences in enrollment criteria it is
Table 3. Transient elastography (TE) and serum fibrosis biomarkers in patients with chronic HCV with and without thalassemia.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A (Chronic HCV and thalassemia)</th>
<th>Group B (Chronic HCV without thalassemia)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TE (n, %)</td>
<td>Patients with SVR (N=36)†**</td>
<td>P</td>
</tr>
<tr>
<td>≤ 7 kPa</td>
<td>27 (75)</td>
<td>0.60</td>
</tr>
<tr>
<td>≥ 7 kPa &lt; 15 kPa</td>
<td>6 (17.6)</td>
<td>0.61</td>
</tr>
<tr>
<td>≥ 15 kPa</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PIINP (ng/mL)</td>
<td>50.3 ±11.2</td>
<td>0.24</td>
</tr>
<tr>
<td>HA (ng/mL)</td>
<td>179.16 ±29.9</td>
<td>0.04*</td>
</tr>
<tr>
<td>YKL-40 (pg/ml)</td>
<td>182.47 ±26.0</td>
<td>± 25.1</td>
</tr>
</tbody>
</table>

* Patient with chronic HCV and thalassemia who achieved SVR: N=36: 13 PEG-IFN SVR/ 23 DAAs SVR. † Non-responders/Not treated chronic HCV and thalassemia patients: 8 patients ineligible for therapy, 3 patients did not tolerate therapy and 5 patients non-responders to PEG-IFN and DAAs regimen. ‡ Patient with chronic HCV without thalassemia who achieved SVR: N=61: 23 PEG-IFN SVR/ 38 DAAs SVR. ‡ Non-responders/Not treated chronic HCV without thalassemia patients: 17 not eligible or discontinued PEG-IFN/RBV NR, 5 not responding to DAAs. Values are N (%) or mean ± SD. ‡P-values from Fisher’s exact test for categorical variables. TE: transient elastography, PIINP: N-terminal procollagen III propeptide, HA: hyaluronic acid.

Table 4. Correlations between TE measurements and parameters of fibrosis in thalassemia patients with chronic HCV (Group A) and patients with chronic HCV without thalassemia (Group B).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Concomitant chronic HCV and β-thalassemia (Group A)</th>
<th>Chronic HCV without thalassemia (Group B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver biopsy Metavir stage Correlation coefficient r (P value)</td>
<td>r = 0.82; (P&lt;0.0001)</td>
<td>r = 0.69; (P&lt;0.0001)</td>
</tr>
<tr>
<td>Ferritin</td>
<td>r = 0.48; (P = 0.01)</td>
<td>r = 0.12; (P = 0.35)</td>
</tr>
<tr>
<td>Ferriscan (MRI T2)</td>
<td>r = 0.81; (P &lt; 0.0001)</td>
<td>r = 0.14; (P = 0.6)</td>
</tr>
<tr>
<td>LIC</td>
<td>r = 0.327; (P=0.01)</td>
<td>r = 0.04; (P=0.5)</td>
</tr>
<tr>
<td>PIINP</td>
<td>r = 0.58; (P&lt;0.01)</td>
<td>R=</td>
</tr>
<tr>
<td>HA</td>
<td>r = 0.62; (P&lt;0.001)</td>
<td>r = 0.32; (P=0.01)</td>
</tr>
<tr>
<td>YKL-40</td>
<td>r = 0.75; (P&lt;0.0001)</td>
<td>r = 0.47; (P=0.02)</td>
</tr>
</tbody>
</table>

difficult to estimate the outcome of acute HCV in patients with thalassemia and further large studies are required.

Our findings showed that patients with concomitant thalassemia and HCV showed higher tendency to develop chronic HCV and were considered for either PEG-IFN based therapy or DAAs according to the standard of care available at the study time points. The SVR of patients with concomitant chronic HCV and thalassemia to PEG-IFN based therapy was significantly lower than SVR in patients without thalassemia in agreement with previous reports.45-47 In the current study, the poor response of patients with chronic HCV and thalassemia to PEG-IFN based therapy may be explained by frequent occurrence of adverse events such as anemia due to ribavirin induced hemolysis, interferon-induced bone marrow suppression and the higher grading and staging scores observed in the baseline liver biopsies of patients with concomitant chronic HCV and thalassemia due to the dual liver injury caused by chronic hepatitis and hepatic iron overload. Although a lower dose of ribavirin was used in thalassemia patients, ribavirin increased hemolysis that necessitated elevating the blood transfusion demands and increased risk of iron overload. Thus, PEG-IFN/RBV regimen was not an efficient safe therapeutic strategy to manage this patient population. In contrast, our study showed significantly enhanced efficacy and safety of DAAs in treating patients with concomitant chronic HCV genotype 4 and thalassemia in whom significantly high SVR rates have been achieved even in treatment-
Figure 3. (A) Liver iron content (LIC) in patients with acute HCV and thalassemia measured by MRI, patients with chronic HCV and thalassemia during PEG-IFN based therapy and DAAs (B): Correlation between liver iron content (LIC) and fibrosis progression rate patients with chronic HCV and thalassemia. (C) Ferritin levels in patients with acute HCV without thalassemia; (D) Correlation between LIC and fibrosis progression rates in patients with acute HCV without thalassemia.

3a: In patients with acute HCV and thalassemia, the baseline LIC detected by MRI (R2) was elevated during the acute phase. LIC levels increased during PEG-IFN/RBV therapy but not with DAAs. 3b: A direct linear correlation was detected between the liver iron content levels and fibrosis progression rate measured as fibrosis units/year. 3c: In patients with acute HCV without thalassemia, the baseline LIC detected by MRI (R2) was elevated during the acute phase. LIC levels increased during PEG-IFN/RBV therapy but not with DAAs. 3d: A direct linear correlation was detected between the liver iron content levels and fibrosis progression rate measured as fibrosis units/year.
The fibrosis progression rates detected in our chronic HCV patients without thalassemia were comparable to those in previous reports. However, patients with concomitant HCV and thalassemia showed a significantly high progression of fibrosis rates which were similar to those reported in patients with HIV or HBV or S. mansoni coinfections and almost one third of this cohort had established cirrhosis by the end of the study. The accelerated hepatic fibrosis is probably attributed to the cumulative effect of HCV induced liver injury and the increased iron overload particularly in inadequately chelated patients, and that was shown in this study and other studies by the direct correlation between LIC and fibrosis progression rates. The accelerated rates of liver fibrosis and high incidence of liver cirrhosis in this patient population highlight the importance of early detection of HCV infection and prompt treatment with DAAs to prevent advanced liver disease with its complications.

Our results showed not only comparability of TE and serum fibrosis markers with the liver biopsy results but also the capability of TE and fibrosis markers in non-invasive monitoring of liver fibrosis progression in patients with thalassemia and HCV in accordance with previous studies. Our study also showed that hepatic iron levels did not interfere with the TE results suggesting that TE alone or in combination with serum fibrosis biomarkers particularly markers measures a quantitative liver fibrosis parameters such as which may be used in follow-up of this patient population.

In the current study, three patients with concomitant HCV and thalassemia died due to cardiac causes and other two due to hepatocellular carcinoma (HCC). Besides early treatment of thalassemia patients with chronic HCV, close monitoring of patients (including those who achieved SVR) for HCC, iron overload and cardiac status, is mandatory.

The current study has several strengths that include the longitudinal prospective study design; the long follow up which stretched for 14 years, the reasonable sample size, the inclusion of patients with acute HCV with and without thalassemia, the comprehensive investigations performed and inclusion of the antiviral regimen available through the study. The limitation of the present study is enrolling patients infected with HCV genotype 4 only because this is the prevalent genotype in Egypt.

Conclusions. Thalassemia patients have low rates of spontaneous eradication of HCV infection and the majority develop chronic HCV. Liver fibrosis is accelerated in thalassemia patients with chronic HCV due to the impact of HCV induced liver injury and iron overload state in thalassemia patients. Direct-acting antiviral agents are highly effective and safe in treating naïve and experienced thalassemia patients with chronic HCV. Therefore, early diagnosis and treatment of this with DAAs is recommended to prevent cirrhosis and hepatocellular carcinoma which are important causes of mortality in this patients population.
References:


https://doi.org/10.1097/00005792-197705000-00002

PMid:8789279

https://doi.org/10.1016/j.ejrad.2006.11.012

PMid:17166881

https://doi.org/10.1002/hep.510240201

PMid:8690560

https://doi.org/10.1016/S0140-6736(96)07642-8

https://doi.org/10.1046/j.1365-2893-2002.00340.x

PMid:11876793

https://doi.org/10.1016/j.jhep.2013.11.016

PMid:24291240

https://doi.org/10.1016/j.jhep.2017.06.002

PMid:29743790 PMCID:PMC5938522

https://doi.org/10.1111/apt.14197

PMid:28660640

https://doi.org/10.4084/mjhid.2017.003

PMid:28101309 PMCID:PMC5224816

https://doi.org/10.1007/s00277-017-0129-9

PMid:28873336

https://doi.org/10.1007/s00277-015-2403-z