Seroprevalence and genotyping of hepatitis C virus in multiple transfused Jordanian patients with β-thalassemia major

Multipl transfüzyon uygulanan β-talasemi majörlü Ürdünlü hastalarda hepatit C virüsünün seroprevalansı ve genotiplemesi

Suleimman Ahmad Al-Sweedan[1], Said Jaradat[2], Khitam Amer[2], Wail Hayajneh[1], Hazem Haddad[2]

[1]*Department of Pediatrics, Jordan University of Science and Technology, Irbid, Jordan*

[2]*Department of Genetics, Jordan University of Science and Technology, Irbid, Jordan*

Abstract

Objective: The main objectives of this study were to investigate the prevalence of hepatitis C virus (HCV) among patients with β-thalassemia major and to determine the most prevalent genotype for this virus.
Materials and Methods: One hundred twenty-two β-thalassemia major patients who were previously diagnosed at the molecular level were included. All plasma samples were tested for the presence of antibodies by ELISA. Real-time polymerase chain reaction (PCR) was used in the quantitation the HCV RNA viral loads, and consequently, patients with high virus titer were genotyped by the linear array.
Results: Forty of the patients were anti-HCV positive. The prevalence of anti-HCV was significantly higher in patients who received blood transfusion before 1993 (83.7%) than in those who received it after 1993 (16.3%) (p=0.000). β-thalassemia major patients with HCV infection had significantly higher rates of elevated aspartate aminotransferase (54.4% vs 40.5%, p=0.045) and alanine aminotransferase (72.47% vs 37.47%, p=0.00) and of splenectomy (54.8% vs 45.2%, p=0.004) than β-thalassemia major patients without HCV.
Conclusion: HCV genotype 4 is the commonest genotype in multi-transfused patients with β-thalassemia major in Jordan. *(Turk J Hematol 2011; 28: 47-51)*

Key words: Virology, transfusion, thalassemia

Received: August 7, 2009 *Accepted: February 22, 2010*

Özet

Amaç: Bu çalışma kapsamında, β-talasemi majörlü hastalarda HCV prevalansının araştırılması ve bu virüsün söz konusu hastalar arasında en yaygın genotipinin belirlenmesi amaçlanmaktadır.
Yöntem ve Gereçler: Önceden moleküler düzeyde tanı konan 122 β-talasemi majörlü hasta çalışmaya alınmıştır. Tüm plazma numuneleri antikor varlığına yönelik olarak ELISA ile test edilmiştir. HCV RNA

Address for Correspondence: MD, MS, FAAP. Suleimman Al-Sweedan,Pediatric Hematology/Oncology/BMTKing Abdalla University HospitalFaculty of MedicineJordan University of Science & Technology, Irbid, Jordan Phone: +0799051255 E-mail: sweedan@just.edu.jo

doi:10.5152/tjh.2011.05

viral yükler ve dolayısıyla doğrusal dizilim ile genotiplenen yüksek virüs titreli hastalar gerçek zamanlı PCR kullanarak belirlenmiştir.
Bulgular: Hastaların 40'ı anti-HCV pozitif idi. Anti-HCV prevalansı, 1993'ten önce kan transfüzyonu uygulanan hastalarda (%83.7), 1993'ten sonra uygulananlara (%16.3) kıyasla anlamlı derecede daha yüksek idi (p=0.00). HCV enfeksiyonlu β-talasemi majörlü hastaların Aspartat aminotransferaz (%54.4; %40.5, p= 0.045) ve Alanin aminotransferaz (%72.47; %37.47, p= 0.00) düzeyleri HCV enfeksiyonsuz β-talasemi majörlü hastalarınkine kıyasla anlamlı derecede daha yüksek olup, bu gruptaki splenektomize hastaların sayısı anlamlı derecede daha yüksek idi (%54.8; %45.2, p= 0.004).
Sonuç: Ürdün'de, multipl transfüzyon uygulanmış β-TM'li hastalarda genotip 4, en yaygın genotiptir.
(Turk J Hematol 2011; 28: 47-51)
Anahtar kelimeler: Viroloji, transfüzyon, talasemi

Geliş tarihi: 07 Ağustos 2009 *Kabul tarihi: 22 Şubat 2010*

Introduction

Beta-thalassemia is an autosomal recessive disorder and occurs because of the absence or reduced synthesis of the beta globin chains [1]. Patients with beta-thalassemia major (β-TM) suffer from numerous complications such as massive hepatosplenomegaly, dental problems, leg ulcers, and high risk for acquiring blood-transmitted infections such as hepatitis B virus (HBV), human immunodeficiency virus (HIV) and particularly hepatitis C virus (HCV) [2,3]. According to estimates by the World Health Organization, 170 million people are infected with HCV worldwide [4]. HCV genome encodes a single poly protein of 3000 amino acids; it is cleaved post translationally to yield at least 10 structural and non-structural proteins [5]. Sequence comparisons of the virus led to the identification of at least six major genotypes [6], and these differ in nucleotide sequence by more than 30% over the complete virus genome. Additionally, there are more than 50 subtypes, which also differ in nucleotides sequence by more than 20% [7]. Genotype 4 is found most commonly in the Middle East [8]. Genotyping and sub-typing for HCV are not only required for therapy initiation and monitoring, but they also assist in vaccine development [9,10]. Studies have shown that HCV genotypes 1 and 4 are more resistant to treatment with pegylated interferon and ribavirin than genotypes 2 and 3 [11].

A study conducted in Jordan among patients on regular hemodialysis showed that the prevalence of HCV infection was correlated with the history of blood transfusion before the introduction of anti-HCV screening in Jordanian blood banks in 1993 [12]. HCV genotype 1a was found to be the predominant sub-type among blood donors and Jordanian hemodialysis patients as well as other Middle Eastern countries including Lebanon, Turkey, Cyprus, and Syria [13,14]. HCV genotype 4 is the most prevalent in Saudi Arabia, Egypt, Yemen, and Bahrain [15]. Patients with hemoglobinopathies have been excluded from large studies of therapy for HCV, particularly studies that include ribavirin, because of the associated blood hemolysis [16]. Both ribavirin and interferon drugs were tested on thalassemic patients with HCV infection and were shown to be effective [17]. The main objectives of this study were (i) to investigate the prevalence of HCV among Jordanian patients with β-TM, and (ii) to determine the most prevalent genotype for this virus.

Materials and Methods

A hospital-based study was carried out on 122 patients pre-diagnosed with β-TM based on hemoglobin electrophoresis and molecular DNA analysis between April -December 2008. Identification of β-TM for over 150 patients, including the patients of this study, was achieved by polymerase chain reaction (PCR) amplification of beta-globin genes and direct DNA sequencing of amplified genomic DNA.

All patients receive regular blood transfusions every 2-4 weeks in Princess Rahmah Governmental Hospital, Irbid, Jordan. All study subjects provided written informed consent or consent was obtained from their guardian in the case of minors, and the study was approved by the Institutional Review Board of Jordan University of Science and Technology. Blood samples were drawn from patients before the scheduled transfusion, and

plasma was separated from whole blood and stored at -80°C until analysis. All plasma samples were tested using the GENEDIA ELISA 3.0 kit (Green Cross Medical Science Corp, Korea) for the presence of antibodies directed against four HCV genomic regions: Core, NS3, NS4, and NS5.

Total RNA was extracted from ELISA-positive samples by the Ribo-Virus spin column extraction kit (Saccace, Italy) according to the manufacturer's specifications. Quantification of the viral load (HCV-RNA) in serum was done by amplification method using the HCV Real-TM Quant MX kit (Sacace, Italy) according to the manufacturer's specifications. The emission of a reporter dye probe specific for HCV, which is proportional to the amount of HCV RNA in the starting sample, was measured using MX4000™ (Stratagene, USA).

Samples with high viral loads were genotyped using the linear array HCV genotyping test kit, version 2.0 (Roche Molecular System) according to manufacturer's specifications. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS, version 16.0) software. Differences between β-TM patients with and without HCV infection were analyzed using the t-test and chi-square test where applicable. Statistical significance was considered at a p value <0.05.

Results

A total of 122 β-TM patients (67 males, 55 females) were studied, and their ages ranged from 2-40 years old (mean age: 14.5 years). A total of 12 different mutations were detected, with the IVS1-110 (G>A) mutation found to be the more frequent among Jordanians (manuscript in preparation). Forty patients were anti-HCV positive. The prevalence of anti-HCV was significantly higher in patients who received blood transfusion before 1993 (83.7%) than in those who received it after 1993 (16.3%) ($p=0.00$) (Table 1). β-TM patients with HCV infection had a significantly higher rate of elevated aspartate aminotransferase (AST) (54.4% vs 40.5%, $p=0.045$), alanine aminotransferase (ALT) (72.47% vs 37.47%, $p=0.00$) and of splenectomy (54.8% vs 45.2%, $p=0.004$) than β-TM patients without HCV (Tables 1, 2). Factors with no statistical significance between the two groups were gender, hemoglobin level, ferritin level, drug intake, and alkaline phosphatase level (Tables 1, 2).

Twenty of 40 samples with ELISA-positive test had high viral loads using the real time PCR assay. HCV genotype 4 was found in 12 patients with high viral titer. Two patients refused to participate in completing the study and genotyping array failed for the other six samples (3 of the failed samples were from patients under prolonged treatment with interferon and ribavirin).

Discussion

Several studies have shown that patients with β-TM receiving chronic blood transfusions have a higher prevalence of chronic HCV infection, particularly if transfused before HCV serological testing became available [18]. Patients with hemoglobinopathies, mainly with thalassemia, have traditionally been excluded from large studies of therapy of HCV [19], particularly studies that include ribavirin because of the associated hemolysis leading to an increase in transfusion requirement, iron accumulation and the risk of iron-related toxicities [20].

A study done among 142 HCV-infected Lebanese thalassemic patients identified HCV genotype 4 as the predominant genotype among thalassemic patients, which is in agreement with our results, and this confirms the predominance of HCV genotype 4 in our country and perhaps in the Middle East [21]. Another study involving 104 thalassemic patients in Thailand showed that prevalence of HCV infection among Thai thalassemic patients was 20.2% [2], while a higher prevalence of HCV infection was observed in our study, with a frequency of 32.8%. Wanachiwanawin et al. [2] showed that patients with anti-HCV antibodies had significantly higher levels of serum AST and ALT than patients without anti-HCV antibodies ($p=0.021$ and 0.017, respectively), in agreement with our results, which revealed that HCV-infected patients had significantly higher levels of ALT and AST than patients without anti-HCV ($p=0.045$ and 0.000, respectively).

A study done among 283 Jordanian hemodialysis patients showed that 98 (34.6%) patients were anti-HCV-positive by ELISA, two HCV genotypes (1 and 4) were identified and HCV genotype la was predominant, whereas in our results, only genotype 4 was identified, and in six samples the genotype arrays failed (3 of the samples were from patients under extended treatment with interferon and ribavirin).

This may be explained by the presence of a new variant or a mixed infection that can not be detected using this genotyping kit [22].

In a study done in Iran in 2006 among 732 patients with β-TM and141 (19.3%) patients who were anti-HCV positive showed that older age (p=0.001), longer transfusion duration (p=0.000) and higher serum ferritin level (p=0.002) were significantly associated with a higher prevalence of HCV [23]. These results are in agreement with our results except for the ferritin level, since we determined no statistical significance for the association between ferritin level and HCV infection. However, our study showed that splenectomized β-TM patients have a higher prevalence of HCV infection. Splenectomized patients are probably heavily transfused with blood before splenectomy due to hypersplenism.

In conclusion, HCV genotype 4 is the commonest genotype in multi-transfused patients with β-TM in Jordan. Considering the possibilities of HCV mixed genotype among patients with thalassemia, accuracy and precision should be an important concern in the detection of genotypes, which might explain the genotyping failure in six patients with high viral load.

Table 1. Age discrimination and laboratory findings of patients

	HCV-infected	HCV-not infected	p value
Age (years) (Mean±SD)	19.08±6.03	12.1±7.5	<0.001
ALT (U/L)	72.47	37.47	<0.001
AST (U/L)	54.4	40.50	0.045
ALP (U/L)	209.12	213.04	0.90
Ferritin (mg/dl)	2131.95	2013.68	0.69
Hb (g/dl)	8.76	8.79	0.86

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; Hb: Hemoglobin

Table 2. Risk factors for HCV infection other than blood transfusion in β-TM patients

Characteristic	HCV-infected patients (%)	HCV-not infected (%)	p value
Surgery	44.7	55.3	0.062
Deferoxamine intake	38.4	61.6	0.58
Splenectomy	54.8	45.2	0.004
Family history of β-TM	37.5	62.5	0.76
Gender Male	33.3	66.7	0.83
Female	34.6	65.4	

Conflict of interest statement

None of the authors of this paper has a conflict of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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