Original Article

Prevalence of Hepatitis B, Hepatitis C, Significant Liver Fibrosis and Its Predictors in Adult Thalassemia Patients who Receive Blood Transfusion

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Background: Thalassemia is a common hereditary hematologic disease requiring frequent blood transfusion that increases patient vulnerability to viral hepatitis B and C.

Objective: To investigate the prevalence of hepatitis B virus (HBV) infection, hepatitis C virus (HCV) infection, and clinically significant liver fibrosis in adult thalassemia patients who have been receiving blood transfusion, and to identify factors associated with liver fibrosis.

Materials and Methods: Patients with thalassemia major and thalassemia intermedia were included. HBV and HCV markers were tested, and patients who were anti-HCV-positive were tested for HCV viral load and HCV genotype. All patients underwent liver transient elastography (TE), and the result was classified as significant fibrosis (≥7.1 kPa) or cirrhosis (>12.5 kPa).

Results: One hundred and fifty-eight patients (36% male, mean age 38 years) were included. All patients had a history of blood transfusion with a mean life-time transfusion of packed red blood cells of 215 units. Prevalence of HBV and HCV was 1.3% and 5.6%, respectively. The mean TE was 7.9 kPa (range 2.4 to 69.1). Fifty-six patients (34%) had significant liver fibrosis, and 18 (11.4%) had cirrhosis. Factors that correlated with significant liver fibrosis were male gender (odds ratio [OR] 3.4, 95% confidence interval [CI] 1.6 to 7.3), serum ferritin ≥1,000 ng/mL (OR 3.4, 95% CI 1.5 to 7.6), and abnormal aspartate aminotransferase (AST) (OR 2.9, 95% CI 1.3 to 6.4). Presence of HBV or HCV was not significantly associated with significant fibrosis.

Conclusion: Prevalence of HBV in thalassemia patients who receive blood transfusion was comparable to general population, but the prevalence of HCV was higher. About half of the patients had significant fibrosis. Factors associated with significant fibrosis were male gender, serum ferritin ≥1,000 ng/mL, and abnormal AST.

Keywords: Blood transfusion, Prevalence, Hepatitis B, Hepatitis C, Liver fibrosis, Thalassemia

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Thalassemia is one of the most common forms of inherited hemolytic anemia. Although this disease has a global presence, thalassemia has a high prevalence in Southeast Asia and the Middle East. In Thailand, it was reported that thalassemia affects about 1% of the population or about 700,000 people[(1)]. There are a variety of hemoglobinopathies in Thailand, including homozygous beta thalassemia, beta thalassemia/hemoglobin E, alpha-thalassemia, and hemoglobin Constant Spring (CS). Blood transfusion is the mainstay treatment for thalassemia, especially thalassemia major. Frequent blood transfusion carries with it a risk of acquiring hepatitis B virus (HBV) and hepatitis C virus (HCV) infection. However, this risk was much higher before 1992, which is when routine hepatitis C screening of blood donors was implemented. Symptomatic anemia is the major problem in thalassemia patients because it can impair growth, and the anemia itself results in more iron absorption. This problem together with frequent blood transfusion will eventually result in iron overload. HBV and/or HCV infection and iron overload can lead to more progressive liver disease, including cirrhosis and hepatocellular carcinoma (HCC). The hazard ratio for death was reported to be significantly higher in thalassemia patients with cirrhosis[(2)].

The prevalence of HCV infection in Thai thalassemia patients from previous study was as high as 20%, but the sample size was small and there was no information regarding the prevalence of viremic HCV or HCV genotypes. The high prevalence of HCV infection among these patients was due to transfusion-related infection that

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occurred prior to the introduction of universal HCV screening in blood donors[3]. There are six major HCV genotypes, and all of them have a different response to HCV treatment[4]. In Thailand, there are 3 major HCV genotypes, including HCV genotypes 3, 1 and 6[5]. Moreover, there has been no study to assess the extent of liver fibrosis in these patients, and whether the presence of either HBV or HCV contributes to the extent of liver fibrosis. Liver biopsy is the gold standard assessment for liver fibrosis, but it is not accepted by most patients and it is riskier in these severely anemic patients. To assess liver fibrosis, transient elastography (TE) is a noninvasive ultrasound-based imaging modality that measures the degree of liver stiffness, and it can be used in an outpatient setting. The sensitivity and specificity of TE was reported to be 68 to 88% and 66 to 100%, respectively, for the detection of significant liver fibrosis compared to liver biopsy (METAVIR score F>2)[6,7]. Newly developed shear wave elastography (2-D SWE) when combined with an ultrasound machine as an add-on feature showed benefit for classifying liver fibrosis in thalassemia patients[8], but other studies found TE to be a more reliable tool for assessing liver fibrosis, including in thalassemia patients[9,10].

There are relatively few studies in the risk factors for liver fibrosis in thalassemia patients, and most of the studies that have been published thus far studied exclusively beta thalassemia patients, which is a type of thalassemia that is highly prevalent in Mediterranean countries. One study from Italy found the TE value of beta thalassemia patients to be correlated with high ferritin levels, HCV-RNA positivity, regular blood transfusion, alanine transaminase (ALT), gamma-glutamyl transferase (GGT), bilirubin level, previous cholecystectomy, and previous splenectomy[10]. Another study in beta thalassemia patients from Greece found age, AST, ALT, and inflammation on liver biopsy tissue to be risk factors for liver fibrosis[9]. In contrast to the high level of beta thalassemia in Mediterranean countries, Southeast Asia (including Thailand) has more thalassemia varieties, including beta thalassemia/hemoglobin E, alpha-thalassemia, and hemoglobin CS, that require regular blood transfusion[11].

The aims of the present study are to investigate the prevalence of HBV infection, HCV infection, and clinically significant liver fibrosis in adult thalassemia patients who have been receiving blood transfusion, and to identify factors significantly associated with liver fibrosis.

Materials and Methods

Patients and study design

This prospective cross-sectional study included thalassemia patients aged >18 years who regularly visited the Hematology Clinic of the Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand during December 2015 to October 2016. This study was approved by our center's institutional review board (COA No. Si657/2015), and written informed consent to participate was obtained from all patients. The protocol for this study was registered at the ClinicalTrials.gov website (reg. no. NCT02904252). Thalassemia major patients, including homozygous beta thalassemia, beta thalassemia/hemoglobin E disease, or thalassemia intermedia, such as hemoglobin H disease or homozygous hemoglobin CS, with previous history of receiving blood transfusion were eligible for inclusion. Patients with decompensated cirrhosis, HCC, pregnancy, or any condition for which TE is contraindicated were excluded.

Data collection

Baseline demographic and clinical data were collected, including history of blood or blood product transfusion. Blood was then collected for complete blood count (CBC), serum ferritin, liver tests, HBsAg, and anti-HCV test (Roche). Patients found to be anti-HCV-positive were further evaluated for HCV viral load and HCV genotype. All included patients were scheduled to undergo TE. All clinical and laboratory data were retrieved from electronic medical records. Among those found to be anti-HCV-positive, the laboratory data included CBC, iron study (serum ferritin, serum iron, total iron binding capacity, and transferrin saturation), hemoglobin typing, anti-HIV status, liver biochemistry (AST, ALT, albumin, globulin), HBsAg, Anti-HBs, Anti-HCV and HCV RNA, and HCV genotype.

TE was assessed by Fibroscan® 502 Touch using an M probe (Echosens, Paris, France). The evaluation was performed after at least 4 hours of fasting on the same day as the initial blood test by a single experienced operator who was blinded to patient clinical data. From a previous study by Castera et al[9], the cutoff points for significant fibrosis and cirrhosis that correlated with a METAVIR fibrosis score F≥2 and F4 were ≥7.1 kPa and ≥12.5 kPa, respectively.

Statistical analysis

All statistical analyses were performed using SPSS Statistics version 18.0 (SPSS, Inc., Chicago, IL, USA). Continuous variables are reported as mean, standard deviation (SD), and categorical variables are given as number and percentage. Comparison between two groups was performed by using t-test or Mann-Whitney U-test, or Chi-square test or Fisher's exact test for continuous and categorical variables, respectively. The results of logistic regression analysis to identify factors significantly associated with severe fibrosis are presented as odds ratio and adjusted odds ratio-both with 95% confidence interval. A p-value of <0.05 was considered statistically significant for all tests.

Results

There were 158 patients enrolled in the present study. The mean age was 38 years, 57 patients were male (36.1%), and 111 patients (70.3%) had beta thalassemia/hemoglobin E disease. Demographic and clinical data of study patients are shown in Table 1. All patients had history of blood transfusion with a mean lifetime transfusion of packed red cells of 215 units, and 132 (83.5%) patients had been receiving iron chelation therapy. However, 82 patients (51.9%) still had a serum ferritin level greater than 1,000 ng/mL. Sixty-one patients (38.6%) were transfusion-dependent. The definition of transfusion-dependent was requirement

Table 1. Characteristic of the 158 patients at the time of transient elastography examination

Male gender	57 (36.1)
Age (years), mean (SD) (range)	38 (15) (18 to 83)
BMI (kg/m^2), mean (SD) (range)	19.6 (2.5) (11.8 to 28.7)
Thalassemia type	
Homozygous beta thalassemia	7 (4.4)
Beta thalassemia hemoglobin E disease	111 (70.3)
Hemoglobin H disease	13 (8.2)
Hemoglobin H disease with hemoglobin Constant Spring	15 (9.5)
Homozygous hemoglobin Constant Spring	1 (0.6)
AE-Bart's disease	1 (0.6)
AE-Bart's disease with hemoglobin Constant Spring	9 (5.7)
EF-Bart's disease with hemoglobin Constant Spring	1 (0.6)
History of splenectomy	45 (28.5)
Estimated number of PRCs transfused (pack), mean (SD)	215 (291)
Transfusion dependent	61 (38.6)
Iron chelation therapy	132 (83.5)
Duration of iron chelation therapy (years), mean (SD) (range)	5.9 (6.6) (0 to 27)
Laboratory profiles	
Hb (g/dL), mean (SD) (range)	7.2 (1.3) (3.4 to 10.9)
Serum ferritin (ng/ml), mean (SD) (range)	1,795 (1,875) (57 to 8,786)
Serum ferritin ≥1,000 (ng/ml), n (%)	82 (51.9)
AST ≥ULN	74 (46.8)
ALT ≥ULN	52 (32.9)
HBsAg positive	2 (1.3)
Anti-HCV positive	15 (9.5)
HCV RNA positive	9 (5.6)
Anti-HIV infection	
Positive	2 (1.3)
Negative	79 (50)
Unknown	77 (48.7)
TE (kPa), mean (SD) (range)	7.9 (6.8) (2.4 to 69.1)

Data are expressed as n (%) unless specified.
ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMI = body mass index; Hb = hemoglobin; HCV = hepatitis C virus; HIV = human immunodeficiency virus; PRCs = packed red cells; RNA = ribonucleic acid; SD = standard deviation; TE = transient elastography; ULN = upper limit of normal

for regular blood transfusion due to symptomatic anemia, suffering from several complications of anemia, and having a shorter estimated life expectancy[10]. Two patients were HBsAg-positive, and 15 patients were anti-HCV-positive (9.5%). No patient received HCV treatment. Only 9 patients were HCV RNA-positive, which means that only 5.6% of our patients were viremic chronic hepatitis C. Of the 2 patients that were HBsAg-positive, one had low HBV DNA viral load, and the other was already receiving antiviral therapy. Among the 9 patients with viremic HCV, the genotypes of 4 patients were 1a, 1b, 3a, and 6, respectively. The other 5 patients were unclassified (3, 2, 2, 1, and 1, respectively). The result of TE measurement ranged from 2.4 kPa to 69.1 kPa with a mean of 7.9 kPa. Fifty-six patients (34%) had significant liver fibrosis, and another 18 patients (11.4%) had cirrhosis (Table 2).

Logistic regression analysis revealed male gender (odds ratio [OR] 3.4, 95% confidence interval [CI] 1.6 to 7.3), serum ferritin ≥1,000 ng/mL (OR 3.4, 95% CI 1.5 to 7.6), and abnormal AST (OR 2.9, 95% CI 1.3 to 6.4) to be significantly associated with clinically significant liver fibrosis (Table 3). Infection with HBV or HCV did not significantly

associated with liver fibrosis.

Logistic regression analysis revealed male gender (OR 3.4, 95% CI 1.6 to 7.3), serum ferritin ≥1,000 ng/mL (OR 3.4, 95% CI 1.5 to 7.6), and abnormal AST (OR 2.9, 95% CI 1.3 to 6.4) to be significantly associated with clinically significant liver fibrosis (Table 3). Infection with HBV or HCV did not significantly associated with liver fibrosis.

Discussion

The aims of this study are to evaluate the prevalence of HBV infection, HCV infection, and clinically significant liver fibrosis in thalassemia patients who have been receiving blood transfusion. We included a large proportion of transfusion-dependent patients with a high serum ferritin level that well represented thalassemia patients at risk for liver fibrosis, and 39% of our patients were transfusion-dependent.

There were only 2 HBsAg-positive (1.3%) patients, which is comparable to general population in Thailand[12]. This may be the result of the implementation of a universal HBV vaccination program that started more than 27 years ago, and meticulous blood donor screening. There were 15 patients found to be anti-HCV positive (9.5%), but only 9 patients (5.6%) were HCV RNA positive, which is much lower than the 20% prevalence of anti-HCV-positive in Thai thalassemia that was previously reported by Wanachiwanawin in 2003[3]. This difference between studies is likely explained by the implementation of HCV screening in blood donors that started in 1991, and the introduction of nucleic acid amplification technology (NAT) by the National Blood Center of the Thai Red Cross Society that started in 2007[13]. However, the prevalence of viremic HCV in this study was higher than in general population. A recent national survey found the prevalence of viremic HCV to be 0.39% in general population[14]. This difference may be due to HCV infection from frequent blood transfusion, and some may have been exposed before NAT was implemented.

Regarding hepatic fibrosis, we found that 74 patients (46%) had clinically significant fibrosis or cirrhosis, and that among those, 18 (11.4%) had cirrhosis by TE. Patients with significant fibrosis can progress to clinical cirrhosis or HCC[2], which requires regular HCC surveillance. TE is also a useful tool for patient follow-up since it is non-invasive. We found male gender, high serum ferritin (≥1,000 ng/mL), and abnormal serum AST to be significant risk factors for liver fibrosis. However, whether or not male gender is an independent risk factor for liver fibrosis in thalassemia patients has not been conclusively established. A study from Oman found iron overload and male gender (regardless of alcohol consumption) to be independently associated with more significant liver fibrosis in beta thalassemia patients[15], however, the findings from a study conducted in Italy showed no correlation[7]. The lower prevalence of liver fibrosis in females may be due to the protective effect of estradiol. In 2011, a study by Xu et al showed that estradiol treatment in mice reduced serum AST, ALT, hyaluronic acid, and type IV collagen, as well as suppressed hepatic collagen content, which lowered the fibrotic response in the liver[16].

A study of liver fibrosis progression in 233 thalassemia patients after liver transplantation by Angelucci et al found serum ferritin, which is an indirect marker for iron overload, to be a risk factor for liver fibrosis progression[17]. They concluded that in thalassemia patients, iron overload plays an important role in liver fibrosis. In that same study, HCV infection was also an independent risk factor for liver fibrosis progression, but we did not find this correlation in our study. This difference in findings may be due to the fact that we had only 9 cases of HCV infection compared to 112 cases in their study.

Our study showed that high AST correlates with liver fibrosis, and this can be explained by several reasons. High AST in thalassemia patients may be from liver and hemolyzed red cell. Chronic hemolysis indicates more severe thalassemia as the result of more blood transfusion, as well as more iron deposition[18,19]. This observation was also found in previous study from Greece among patients with hemolysis who had increased serum ferritin and AST levels that were found to correlate with liver fibrosis[6]. Moreover, in chronic

Table 2. Transient elastography value in 158 patients

Transient elastography value (kPa)	n (%)
<7.1	84 (53.1)
≥7.1 to 12.4	56 (35.4)
≥12.5	18 (11.4)

Table 3. Significant risk factors of liver fibrosis by logistic regression model

Factors	Crude OR (95% CI)	*p*-value	Adjusted OR (95% CI)	*p*-value
Male gender	3.6 (1.8 to 7.2)	<0.001	3.4 (1.6 to 7.3)	0.002
Serum ferritin ≥1,000 ng/mL	4.6 (2.3 to 9.6)	<0.001	3.4 (1.5 to 7.6)	0.004
AST ≥ ULN	5.0 (2.4 to 10.2)	0.001	2.9 (1.3 to 6.4)	0.009

AST = aspartate aminotransferase; CI = confidence interval; OR = odds ratio; ULN = upper limit of normal

liver disease, an increased AST/ALT ratio indicates advanced fibrosis.

The strengths of the present study include that this is the first study in Southeast Asia to investigate and report risk factors for liver fibrosis in thalassemia patients, especially among beta thalassemia/hemoglobin E disease, which is only found in this region. Moreover, we included a substantial proportion of transfusion-dependent patients with a high serum ferritin level that well represented thalassemia patients at risk for liver fibrosis. The limitations of our study include its cross-sectional design, which means that some data could not be accurately collected, especially type, dosage, and duration of iron chelation. Another limitation is the low prevalence of HBV and HCV, which limited our statistical ability to assess the impact of these infections on clinically significant liver fibrosis.

Conclusion

Among adult thalassemia major and intermedia patients who had been receiving regular blood transfusions, the prevalence of HBV infection was low and comparable to general population. In contrast, the prevalence of HCV was higher than general population, but lower than previous reports. Almost half of adult thalassemia patients had clinically significant liver fibrosis or cirrhosis. Factors significantly associated with clinically significant fibrosis included male gender, serum ferritin ≥1,000 ng/mL, and abnormal AST. Intensive iron chelation therapy and periodic TE may be helpful to identify patients at risk for significant liver fibrosis, and to monitor for HCC.

What is already known on this topic?

Studies in the past showed that thalassemia major and intermedia patients who had received blood transfusion increased the prevalence of HBV and HCV infection, especially HCV can be found as high as 20%. As the result more progressive liver fibrosis is found in thalassemia patients in addition to iron overload. Meticulous blood screening for HBV and HCV as well as HBV vaccination may decrease the risk of getting both viruses.

What this study adds?

Prevalence of HBV in thalassemia major and intermedia patients was low, comparable to that in general population, however, the prevalence of HCV is slightly higher than general population and much lower than previous study. About one-third of thalassemia major and intermedia patients who had received blood transfusion has significant fibrosis. Risk factors of significant fibrosis include male gender, serum ferritin >1,000 ng/mL, and abnormal AST.

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Conflicts of interest

The authors declare no conflict of interest.

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ความชุกของการติดเชื้อไวรัสตับอักเสบบี ไวรัสตับอักเสบซี การเกิดพังผืดในตับ และปัจจัยบ่งชี้ในผู้ป่วยธาลัสซีเมียที่ได้รับเลือด

อวยพร เค้าสมบัติวัฒนา, พัทธดนย์ ศิริวงศ์รังสรรค์, ธีระ ฤชุตระกูล, ทวีศักดิ์ แทนวันดี

ภูมิหลัง: โรคธาลัสซีเมียเป็นโรคโลหิตจางที่เกิดจากพันธุกรรมซึ่งพบได้บ่อย ผู้ป่วยต้องได้รับเลือดซึ่งเพิ่มความเสี่ยงต่อการติดเชื้อไวรัสตับอักเสบบีและซี

วัตถุประสงค์: เพื่อหาความชุกของการติดเชื้อไวรัสตับอักเสบบี ไวรัสตับอักเสบซี และความชุกของการเกิดพังผืดในตับ ในผู้ป่วยโรคธาลัสซีเมียที่มีประวัติเคยได้รับเลือด และหาปัจจัยเสี่ยงของการเกิดพังผืดในตับที่มีนัยสำคัญ

วัสดุและวิธีการ: คัดเลือกผู้ป่วยโรคธาลัสซีเมียเมเจอร์หรืออินเตอร์มีเดียที่มีประวัติเคยได้รับเลือด ตรวจหาไวรัสตับอักเสบบีและซี ผู้ป่วยที่ anti-HCV เป็นบวก จะได้รับการตรวจจำนวนไวรัสและสายพันธุ์ ผู้ป่วยทุกรายจะได้รับการตรวจ transient elastography (TE) และจำแนกเป็นมีพังผืดตับที่มีนัยสำคัญ (≥7.1 กิโลปาสคาล) หรือตับแข็ง (>12.5 กิโลปาสคาล)

ผลการศึกษา: มีผู้เข้าร่วมวิจัยทั้งสิ้น 158 คน เป็นเพศชายร้อยละ 36 อายุเฉลี่ย 38 ปี มีประวัติได้รับเลือดเฉลี่ย 215 ถุง ความชุกของการติดเชื้อไวรัสตับอักเสบบีและซีคือร้อยละ 1.3 และร้อยละ 5.6 ตามลำดับ ค่า TE เฉลี่ยอยู่ที่ 7.9 กิโลปาสคาล (พิสัย 2.4 ถึง 69.1 กิโลปาสคาล) ผู้ป่วย 56 ราย (ร้อยละ 34) มีพังผืดในตับที่มีนัยสำคัญ และ 18 ราย (ร้อยละ 11.4) มีภาวะตับแข็ง ปัจจัยที่เกี่ยวข้องกับการเกิดพังผืดในตับที่มีนัยสำคัญ คือ เพศชาย (3.4 เท่า, ช่วงความเชื่อมั่นร้อยละ 95 1.6 ถึง 7.3), ระดับ ferritin >1,000 นก./มล. (3.4 เท่า, ช่วงความเชื่อมั่นร้อยละ 95 1.5 ถึง 7.6) และค่า AST สูงกว่าปกติ (2.9 เท่า, ช่วงความเชื่อมั่นร้อยละ 95 1.3 ถึง 6.4) การมีไวรัสตับอักเสบบีหรือซีไม่เกี่ยวข้องกับการเกิดพังผืดในตับที่มีนัยสำคัญ

สรุป: ความชุกของการติดเชื้อไวรัสตับอักเสบบีในผู้ป่วยโรคธาลัสซีเมียที่เคยได้รับเลือดเหมือนกับประชากรทั่วไป แต่ความชุกของการติดเชื้อไวรัสตับอักเสบซีนั้นสูงกว่าประชากรทั่วไป ผู้ป่วยประมาณครึ่งหนึ่งมีพังผืดในตับที่มีนัยสำคัญ ปัจจัยที่เป็นความเสี่ยงต่อการเกิดพังผืดในตับที่มีนัยสำคัญ คือ เพศชาย ระดับ ferritin >1,000 นก./มล. และค่า AST สูงกว่าปกติ