Original Article

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Comparative Study of Liver Stiffness Measurement (LSM) in Transfusion Dependent and Non-Transfusion Dependent Thalassemic Patients

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ABSTRACT

Background: Ineffective erythropoiesis coupled with chronic anemia in thalassemic patients may lead to increase gastrointestinal iron absorption which may result in liver fibrosis. Although liver biopsy is the gold standard tool for the diagnosis of liver fibrosis, recent noninvasive tests may also help in the evaluation.

Objective: We aimed to compare the difference of liver stiffness measurement between transfusion dependent and non-transfusion dependent thalassemia. We also assessed the prevalence of significant fibrosis and attempted to identify factors that could be associated with significant fibrosis.

Methods: A cross-sectional assessment of liver fibrosis using transient elastography (TE) was performed in 109 thalassemic patients were seen at the Hematology Unit and the Liver Unit, Ramathibodi Hospital over a 1-year period. Baseline demographic data, anthropometry, type of thalassemia, history of chelation therapy and dose of treatment, and related blood tests (CBC, LFT, ferritin, HBsAg, Anti HCV, Anti HIV) were collected. TE was performed by standard technique. TE ≥ 7.9 kPa was defined as significant fibrosis. Statistical analysis appropriately made using by Student t-test, Wilcoxon rank-sum test, and Spearman correlation or Chi-Square test. Factors with p-value < 0.05 were selected for multivariate analysis.

Results: A total of 109 thalassemic patients (66 transfusion dependent and 43 non-transfusion dependent) were enrolled. Most patients in both groups (77%) were female. The prevalence of significant fibrosis (TE ≥ 7.9) in TDT and NTDT was 24.2% and 18.6%. All patients in TDT group were chelated, comparing with 66% in the NTDT group. The median liver stiffness measurements in TDT vs. NTDT were 5.65 (2.5, 36.8) and 5.35 (3.2, 12.2) (p= 0.535). From univariate analysis of significant fibrosis, the associated factors were male sex, non-chelation, higher serum ferritin, AST, ALT and GGT (p< 0.05). However, ALT level was the only predictive factor for significant fibrosis (OR 1.04, 95 % CI (1.01-1.06), p< 0.05).

Conclusion: The median liver stiffness did not significantly differ between transfusion dependent and non-transfusion dependent thalassemia. The prevalence of significant liver fibrosis (TE ≥ 7.9 kPa) in thalassemia patients was 22%. Serum ALT level was the only factor that appears to be associated with significant liver fibrosis.

Key words : Thalassemia, transient elastography, significant fibrosis

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INTRODUCTION

Thalassemia is a form of hereditary anemia caused by an absent or decreased production of beta-globin chains. The disease is associated with considerable morbidity and mortality[1-6]. The thalassemia syndrome can be divided into two groups: transfusion dependent thalassemia (TDT) and non-transfusion dependent thalassemia (NTDT). TDT patients frequently require blood transfusion, approximately 1 unit of packed red cells every 4 to 6 weeks. Frequent blood transfusion and increased gastrointestinal iron absorption can eventually lead to iron overload. Moreover, in NTDT, iron overload is mainly due to ineffective erythropoiesis and iron absorption via hepcidin. The liver was damaged from free iron in the serum via the lipid peroxidation process, which results in cell death and liver fibrosis.

Liver biopsy is considered the gold standard procedure for evaluating the grade of hepatic inflammation and the stage of fibrosis, and for measuring iron concentration in the liver tissue[7]. However, there are drawbacks in taking a liver biopsy which include sampling error and interobserver variability[8]. In recent years, many non-invasive methods have been developed, including biochemical tests and liver stiffness measurement (LSM) by transient elastography (TE, Fibroscan®)[9]. The later technology has been shown closely related to the degree of liver fibrosis as assessed from liver biopsy. TE has been used in many chronic liver diseases including thalassemia[10]. It is a rapid and non invasive technique to measure liver stiffness. The machine is equipped with a probe consisting of an ultrasonic TE elastography is a reliable non-invasive tool for diagnosing liver fibrosis in thalassemic subjects without iron interference. Serum ferritin is a non-invasive test which is correlated to liver iron concentration in some studies. Lastly, some serum fibrosis markers such as serum hyaluronic acid and transforming growth factor-beta 1 are some novel markers that can accurately detect liver fibrosis.

Previous studies have shown that liver stiffness as measured by TE had a good correlation with liver fibrosis. Mirella Fraquelli *et al.* in a study in adult thalassemia patients found that a cut-off level of ≥ 7.9 kPa was associated with a sensitivity and a specificity of 72% and 84%, respectively[11].

The main aim of our study was to compare liver stiffness measured by transient elastography, in transfusion dependent and non-transfusion dependent thalassemia as and to assess the prevalence of significant fibrosis as well as determining the factors associated with significant liver fibrosis in thalassemic patients.

PATIENTS AND METHODS

Adult thalassemic patients who were seen at the Hematology Unit, Ramathibodi Hospital, between January 1, 2013 and December 31, 2013 were enrolled into the study. Exclusion criteria were pregnancy, fever or infection in the preceding one week, HIV co-infection, acute hepatitis, history of herbal medicine ingestion, and refusal to participate in the study. Detailed demographic and medical history were collected including sex, age, phone number, BW (kg), height (meters), medical co-morbidity, type of thalassemia, history of chelation threrapy, type of blood transfusion and the last date of transfusion. The present study was approved by Ramathibodi Hospital Ethic Committee.

Biochemical testings included aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (TB), gamma glutamyl transferase (GGT), hematocrit (Hct), platelet (Plt), serum ferritin, screening for hepatitis B and hepatitis C (HBsAg, anti HCV) and anti-HIV.

Liver stiffness measurement was performed over the right lobe of the liver through the intercostal spaces with the patient lying in the dorsal decubitus position with the right arm in maximal abduction. The tip of transducer probe was covered with coupling gel and placed on the skin between the ribs at the level of the hepatic right lobe. The operator, assisted by the time-motion ultrasound image, located a liver portion at least 6-cm thick and free of large vascular structures and pressed the probe button to begin the measurements. The measurement depth was between 25-65 mm.

Ten validated measurements were performed. The success rate was calculated as the number of validated measurements divided by the total number of measurements. The results were expressed in kilopascal (kPa) which ranges from 2.5-75 kPa. The median value of successful measurements was kept as representative of liver stiffness. The entire examination lasted less than five minutes. Only procedures with 10 validated measurements with a successful rate of at least 60% and an interquartile range (IQR) less than 30% were considered reliable. The liver stiffness measurement was evaluated by two well-trained nurses who were blinded to clinical data. Two tubes of clot blood were

collected. All patients undertook transient elastography.

Statistical analysis

Continuous variables with normal distribution were reported as mean ±SD. Categorical data were reported as frequency and percentage. Continuous data were compared between two groups using *t*-test or Mann-Whitney rank sum test. The Chi-square test was used for categorical data analysis, where appropriate. To find as association, Spearman correlation was used for continuous variables and Wilcoxon rank-sum test was used for categorical variables. Logistic regression analysis was used for multivariate analysis. Odds ratio (OR) and its 95% confidence interval (CI) were estimated. All analyses were performed using STATA, version 13.0. A p-value < 0.05 was considered to be statistically significant.

RESULTS

Clinical and biochemical characteristics of 109 thalassemic patients were summarized in Table 1. The mean age of patients were 35.9±11.9 years, 77 percent of patients were female. Most of them were lean which BMI around 19.8±2.5 kg/m^2. Sixty-percent of patients were transfusion dependent thalassemia. Most of patients (81.6%) were beta-thalassemia/HbE disease. Median (range) transient elastography overall was 5.5 kPa (2.5, 36.8).

Table 2 display baseline characteristic, blood test and transient elastography between transfusion dependent (TDT) and non transfusion dependent thalassemia (NTDT). In TDT group, mean age (35.3±1.6) is lower than NTDT group (36.9±9.4). Most of them in both groups were female (77%). Most patients in the TDT group had beta-thalassemia/HbE disease concurrent chronic hepatitis B or C infection and had previous chelation therapy. These factors significantly differed from the NTDT group, excepting a significantly lower Hct in the TDT group (22.9±4.4% vs. NTDT (25.6±4.8%). Serum AST, ALT, GGT, TB, and serum ferritin were not significantly different in the two groups. The median of transient elastography measurement between the TDT and the NTDT groups was also not significantly different [5.65 (2.5, 36.8) kPa vs. 5.3 (3.2, 12.2) kPa, p=0.535]. The prevalence of significant fibrosis by TE criteria (≥ 7.9 kPa) was 24.2% and 18.6% in the TDT and the NTDT group, respectively.

Table 3 displays correlations between stiffness measurement and related variables. Male sex and, beta-thalassemia/HbE were significantly correlated with liver stiffness. However, Hct showed an inverse correlation to stiffness score (correlation coefficient, r = -0.284, p=0.003). Others variables including BMI, transfusion type, chelation status, concurrent chronic hepatitis B or C infection, serum AST, ALT, GGT, TB, ferritin, and platelet did not correlate with stiffness score.

Table 4 and Table 5 display correlations between significant liver fibrosis (≥ 7.9 kPa) and related variables. From univariate analysis; male sex, chelation therapy, serum AST, ALT and GGT were significantly associated with liver fibrosis. From multivariate analysis, only serum ALT was an independent factor associated with significant liver fibrosis (OR 1.04, 95% CI 1.016-10.68, p= 0.0001)

Table 1. Main Characteristics of thalassemic patients.

Characteristics	Patients (n =109)
Age (yr)	35.9 ± 11.9
Female, n (%)	84 (77)
BMI (kg/m^2)	19.8 ± 2.5
Transfusion type, n (%)	66 (60)
Beta-thalassemia/ HbE, n (%)	89 (81.6)
Concurrent CHB/CHC infection, n (%)	10 (9.2)
On chelation therapy, n (%)	92 (84.4)
AST (IU/L) [a]	32 (5,184)
ALT (IU/L) [a]	36 (13,166)
GGT (IU/L) [a]	33 (7,320)
Total Bilirubin (mg/dL) [a]	2.4 (0.6,8.8)
Platelet (*10^3 /mm^3) [a]	366 (69,1018)
Ferritin[a]	826 (26,18216)
TE (kPa)	5.5 (2.5,36.8)

[a] = median (range) TDT; transfusion dependent thalassemic patients NTDT; non- transfusion dependent thalassemic patients

DISCUSSION

Previous studies in 13 patients with beta-thalassemia and post- transfusion iron overload and in 56 patients with homozygous beta-thalassemia reported that TE could reliably diagnose advance liver fibrosis[12,13].

To our knowledge, this is the first cross-sectional study comparing liver stiffness measurement between transfusion dependent and non-transfusion dependent

Table 2. Main characteristics of thalassemic patients according to transfusion dependency.

Characteristics	TDT (n =66)	NTDT (n=43)	*p*-value
Age (yr)	35.3 ± 1.6	36.9 ± 9.4	0.50
Female, n (%)	51 (77.2)	33 (76.7)	0.56
BMI (kg/m^2)	19.7 ± 0.29	20 ± 0.41	0.54
Beta-thalassemia/ HbE, n (%)	60 (90.9)	29 (67.4)	0.002
Concurrent CHB/CHC infection, n (%)	9 (13.6)	1 (2.4)	0.04
On chelation therapy, n (%)	66 (100)	26 (60.4)	0.001
AST (IU/L) [a]	33 (5,184)	31 (14,96)	0.53
ALT (IU/L) [a]	36.5 (19,166)	34 (13,149)	0.51
GGT (IU/L) [a]	31.5 (11,320)	36 (7,140)	0.06
Total Bilirubin (mg/dL) [a]	2.5 (0.8,8.8)	2.1 (0.6,7.7)	0.14
Platelet (*10^3 /mm^3) [a]	432 (69,1018)	291 (90-928)	0.51
Ferritin, n (%)			
< 300	13 (19.7)	12 (27.9)	NS
300 - 800	16 (24.2)	13 (30.2)	
800 - 1,500	14 (21.2)	9 (20.9)	
1500 - 2,500	11 (16.6)	3 (6.9)	
>2,500	12 (18.1)	6 (13.9)	
TE [a]	5.65 (2.5,36.8)	5.35 (3.2,12.2)	0.535
TE ≥ 7.9 kPa, n (%)	16 (24.2)	8 (18.6)	0.24

[a] = median (range) TDT; transfusion dependent thalassemic patients NTDT; non- transfusion dependent thalassemic patients

Table 3. Univariate analysis for factors correlated to stiffness measurement.

Characteristics	Stiffness score correlation coefficient (r)	*p*-value
Age (yr)	0.062	0.51
Female	5.15 (2.5,32.8)	< 0.05
BMI (kg/m^2)	-0.060	0.53
TDT	5.65 (2.5,36.8)	0.53
Beta-thalassemia/ HbE	5.8 (2.5,36.8)	< 0.05
Concurrent CHB/CHC infection	5.6 (3.8,10.2)	0.47
Chelation	5.65 (2.5,36.8)	0.54
AST (IU/L)	0.386	< 0.05
ALT (IU/L)	0.356	< 0.05
GGT (IU/L)	0.426	< 0.05
Total bilirubin (mg/dL)	0.134	0.16
Platelet (*10^3 /mm^3)	0.084	0.38
Ferritin	0.163	0.08

thalassemic patients. Our results showed that liver stiffness measurement in the two groups did not significantly differ. In the NTDT group, TE scores were not lower than in the TDT group. In the NTDT group, this could be explained on the basis of ineffective erythropoiesis related to gastrointestinal iron absorption, as iron deposition can cause liver fibrosis.

A recent study in 2010 by Mirella Fraquelli et al reported that TE cut-off score of 7.9 kPa was associated with significant fibrosis, with a sensitivity of 72%

Table 4. Univariate analysis for factors correlated to significant liver fibrosis.

Characteristics	Stiffness score correlation coefficient (r)		*p*-value
	TE < 7.9 kPa	TE ≥ 7.9 kPa	
Age (yr)	36.0 ± 11.6	35.5 ± 1.14	0.84
Female	70 (82.4%)	14 (58.4%)	< 0.05
BMI (kg/m^2)	19.8 ± 2.3	19.9 ± 0.65	0.86
TDT	49 (57.6%)	17 (70.8%)	0.243
Beta-thalassemia/ HbE	69 (81.8%)	20 (83.3%)	0.81
Concurrent CHB/CHC infection	9 (10.5%)	1 (4.17%)	0.33
Chelation	68 (80%)	24 (100%)	< 0.05
AST (IU/L)	30 (5,91)	47 (20,184)	< 0.05
ALT (IU/L)	33 (13,106)	54.5 (22,166)	< 0.05
GGT (IU/L)	29 (7,182)	52.5 (13,320)	< 0.05
Total Bilirubin (mg/dL)	2.4 (0.8,7.9)	2.85 (0.6,8.8)	0.24
Platelet (*10^3 /mm^3)	326 (77-983)	535 (69,1018)	0.32
Ferritin	798 (26,6815)	1497 (180,18216)	0.01

Table 5. Multiple regression analysis for variables associated with TE values.

Factors	Odd ratio	SE (Std error)	*p*-value	95%CI
ALT	1.042	0.013	< 0.05	1.016-10.68

and a specificity of 84%[10]. In our study, the prevalence of significant liver fibrosis as defined by TE criteria was 24.2% in the TDT and 18.6% in the NTDT group. In terms of stiffness score correlation coefficient, we found that lower levels of hematocrit were related to higher degrees of liver fibrosis. Patients in this group often had regular transfusion resulting in liver fibrosis. The stiffness score was also correlated to serum AST, ALT and GGT values which, could reflect inflammation of the liver.

Interestingly in thalassemic patients, only serum ALT level was related to liver stiffness score, and could predict significant liver fibrosis.

In chronic liver disease of different etiologies, chronic hepatitis C infection is the most relevant factor influencing liver stiffness[12]. Our study did not show this association, however, which could be due to small number of patients with concurrent hepatitis infection (found only 9.2% or 10 patients).

Limitation of this study was related to the lack of liver biopsy histopathology. In current clinical practice, liver biopsy is not always needed unless clinical or biochemistry information so dictated. Recent valid non-invasive techniques such as T2*MRI or SQUID have shown the amount of iron overload and fibrosis to be more accurate.

Conclusions

The median liver stiffness measurement did not significantly differ between transfusion dependent thalassemia and non-transfusion dependent thalassemia. The prevalence of significant liver fibrosis (TE ≥ 7.9 kPa) in thalassemic patients was 22%. Serum ALT level was the only factor associated with significant liver fibrosis.

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