List of Publications

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1) Chueamuangphan N, Wongtheptien W, Nawarawong W, Sukornthasarn A, Chuncharunee S, Tawichasri C, Patumanond J. Clinical indicators for pulmonary arterial hypertension in thalassemia. J Med Assoc Thai. 2012;95(1):16-21.

2) Chueamuangphan N, Wongtheptian W, Patumanond J, Sukornthasarn A, Chuncharunee S, Tawichasri C, Nawarawong W. Effect of acetylsalicylic acid on thalassemia with pulmonary arterial hypertension. Int J Gen Med. 2014;7:525-9.

3) Chueamuangphan N, Patumanond J, Wongtheptien W, Nawarawong W Sukornthasarn A, Chuncharunee S, Tawichasri C. Benefits of chronic blood transfusion in hemoglobin E/β thalassemia with pulmonary arterial hypertension. Int J Gen Med. 2014;7:411-6.

Appendices

Appendix A

Philosophical context of

Clinical epidemiology design in this thesis

Philosophical context of clinical epidemiology design in this thesis

Research questions included in this thesis

1. What are clinical indicators of pulmonary arterial hypertension in thalassemia?
2. Is antiplatelet (acetylsalicylic acid) effective for treatment pulmonary arterial hypertension in thalassemia?
3. Do pulmonary artery systolic pressure decrease after chronic blood transfusion in thalassemia intermedia with pulmonary arterial hypertension?

Research titles for publication

Study I

Clinical indicators for pulmonary arterial hypertension in thalassemia

Study II

Effect of acetylsalicylic acid on thalassemia with pulmonary arterial hypertension

Study III

Benefits of chronic blood transfusion in hemoglobin E/β thalassemia with pulmonary arterial hypertension

1. Theoretical design

An etiologic research[1,2]

The first question is under a philosophical context of etiologic research and the second and third questions is under a philosophical context of therapeutic research. Etiologic research answers the questions “What are risk factors of event occurrence?”

Determinant(s) of risk factors of event occurrence are called “risk factors or etiologic research”. Occurrence relations are classified into causal and non-causal (descriptive) research. Causal research explains causal effect relationship between a determinant and event occurrence, while descriptive research explains event from determinants without interest any

relationship between cause and effect. The occurrence relations of first researche is non-causal elements, and those of the last two researchs are causal element.

The occurrence relation can be written as

Descriptive research (exploratory)

Condition Y = f ($x1+x2 \dots x_k$)

Example: PAH = f (demographic characteristics + signs + laboratory findings)

PAH: pulmonary arterial hypertension

Causal research (explanatory)

Event (y) = f (cause x | confounders)

Example: PAH = f (ASA | age + type of thalassemia + ……)

ASA: acetylsalicylic acid

1.1 Clinical indicators for pulmonary arterial hypertension in thalassemia

This study is an etiologic descriptive research. The objective of the study was to explore factors related to PAH in thalassemia The occurrence relation is shown as follows:

Event (PAH) = f (age + gender + thalassemia type + splenectomy + red cell transfusion + functional class + right heart failure + Hb level + serum ferritin level)

1.2 Effect of acetylsalicylic acid on thalassemia with pulmonary arterial hypertension

This study is a therapeutic causal research. The objective of the study was to determine the mean differences of pulmonary artery systolic pressure (PASP) between ASA and non-ASA group. The occurrence relation is shown as follows:

Event (mean difference) = f (ASA | propensity score)

propensity score: thalassemia type, splenectomy, O2 saturation, functional class, red cell transfusion, right heart failure, Hb level, platelet, nucleated red cell, serum ferritin level, baseline PASP and cardiographic findings

1.3 Benefits of chronic blood transfusion in hemoglobin E/β thalassemia with pulmonary arterial hypertension

This study is a therapeutic causal research. The objective of the study was to determine the mean differences of pulmonary artery systolic pressure (PASP) between chronic blood transfusion and occasional transfusions. The occurrence relation is shown as follows:

Event (mean difference) = f (chronic blood transfusion | propensity score)

propensity score: thalassemia type, splenectomy, O2 saturation, functional class, red cell transfusion, right heart failure, Hb level, platelet, nucleated red cell, serum ferritin level, ASA, baseline PASP and cardiographic findings

2. Data collection design

2.1 Study setting and period

All studies in this thesis were conducted at Department of Medicine, Chiang Rai Hospital, Chiang Rai, Thailand. This is a regional hospital in the northern part of Thailand.

In the first study, all patients with thalassemia disease, who were treated at the hematology outpatient clinic between January 2005 and June 2010 were included. The second study, all new cases of thalassemia with PAH from January 2007 to January 2012 were included. The medical record were retrospectively reviewed by the researcher.

The third study, All cases of E/β-Thal with PAH attending the clinic between June 2011 and November 2012 were included.

2.2 Study domain

Study I

Patient domain in study I is all adult (≥ 15 years) patients with thalassemia disease, who were treated at the hematology outpatient clinic, The exclusion criteria are patients with clinical evidence of other secondary causes of PAH, including HIV infection, collagen vascular diseases and chronic obstructive airway diseases. Acquired heart disease associated with pulmonary venous hypertension; mitral valve disease, congenital heart disease and

hyperthyroidism were also excluded. No patients used the following classification of drugs: antiplatelet,anticoagulants, calcium channel blockers or vasodilators.

Study II

Patient domain in study II is all new cases of adult thalassemia with PAH. The exclusion criteria are patients with other causes of PAH or who used other antiplatelets and/or anticoagulants.

Study III

Patient domain in study III is all adult cases of E/β-Thal with PAH. The exclusion criteria are patients who had clinical evidence of other secondary causes of PAH and serum ferritin level ≥ 2,500 μg/dL.

2.3 Study design

Study I

Study design was etiologic research. Data collection design is extended crossectional study. The primary outcome of this study is the clinical risk indicators of PAH (PASP ≥ 35 mmHg by Doppler echocardiography (ECHO)).

Study II

Study design was therapeutic causal research. Data collection design is retrospective cohort study.

Study III

Study design was therapeutic causal research. Data collection design is non randomized clinical trial.

2.4 Data collection process

The studies were conducted after approval from the Institutional Review Board.

Study I and II

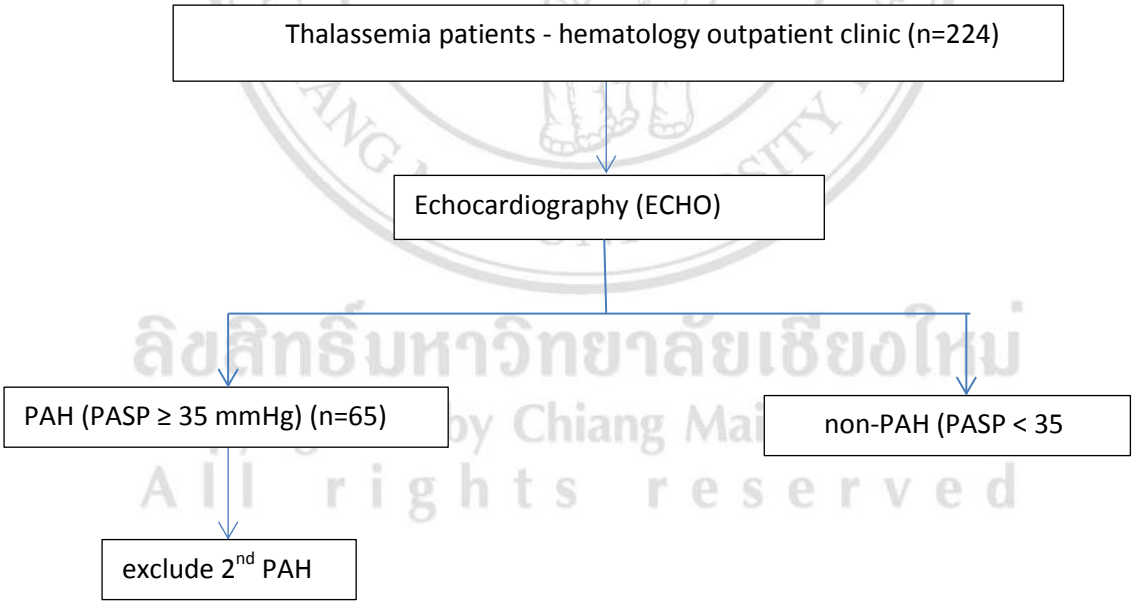
All medical records of the patients who were diagnosed thalassemia disease were reviewed. PASP, estimated by ECHO, was performed on all patients by the same cardiologist.

Study III

All E/β-Thal with PAH patients who met the inclusion criteria were visited by the attending physician. The patients were classified into one of two groups according to their preference. Group 1 patients were those with a preference for chronic blood transfusions. Group 2 patients, comprising those patients who indicated a preference for occasional blood transfusions. PASP and 6-minute walk distance (6MWT: chest team) were measured at baseline and at 6 and 12 months. The case record forms were completed by the researcher.

2.5 Study flow

Study I



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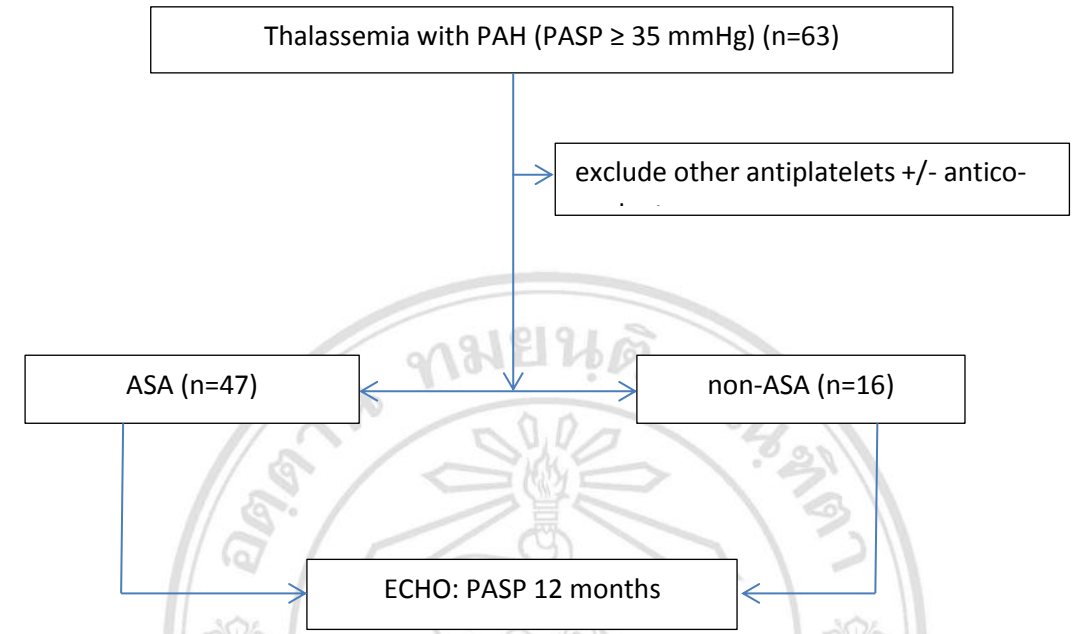
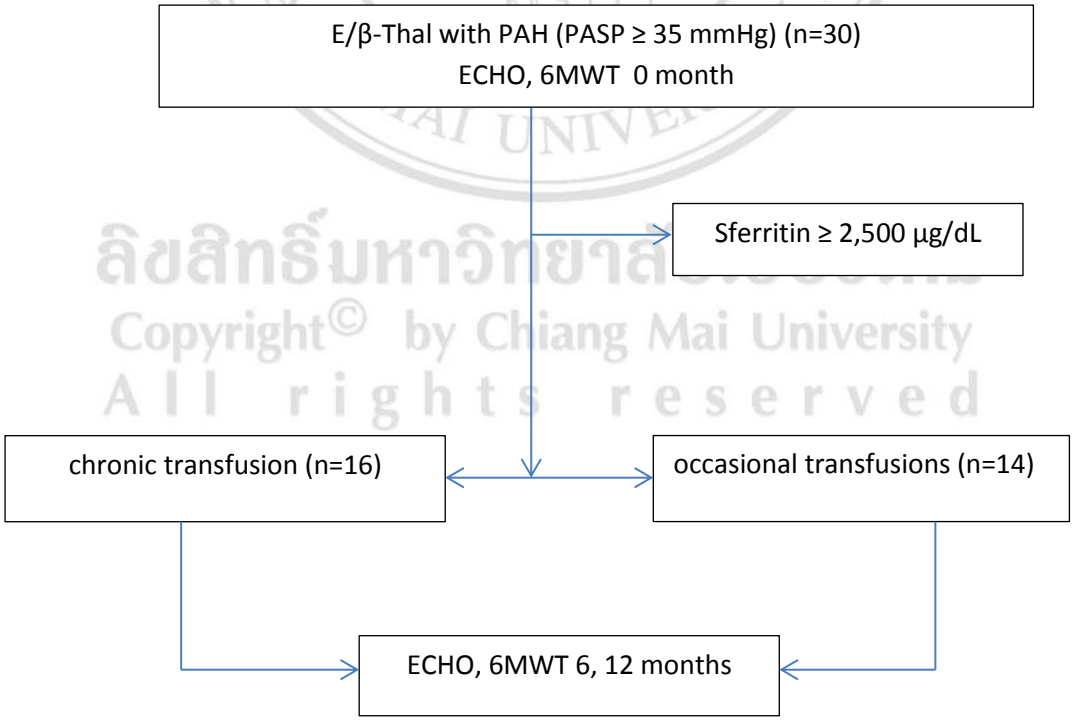


Study III



3. Data analysis design

3.1 An exploratory research[1,2]

Study I is descriptive theoretical design or an exploratory research and primary outcome is the associated factors/indicators of PAH in thalassemia. The characteristics related to disease (presented with odds ratios [OR] and their 95% confidence interval [CI] were explored and tested with multivariable logistic regression.

3.2 An explanatory research[1,2]

Study II and III are explanatory theoretical design and therapeutic research and primary outcomes are mean differences of PASP (and 6MWT in study III) between study and control group. The statistical analysis included three-stage analysis as follows:

Step 1: Student t-test or rank sum test was used to compare continuous data between groups and Fisher's exact test was used to compare categorical data between both groups.

Step 2: Propensity score adjustment was used to control confounding by ASA/chronic blood transfusion indication and contraindication. Propensity scores for receiving ASA/ chronic blood transfusions versus non-ASA/occasional transfusions were calculated from a logistic regression model that estimated the likelihood of receiving ASA/chronic blood transfusions based on the observed patient characteristics.

Step 3: Gaussian and an exponential risk regression were carried out. Univariable and multivariable regression analyses were used to evaluate the effects of ASA/chronic blood transfusions.

Data presentation will be effects of each variable which are mean differences, and their 95% confidence intervals (CI).

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Appendix B

Chueamuangphan N, Wongtheptien W, Nawarawong W, Sukornthasarn A, Chuncharunee S, Tawichasri C, Patumanond J. Clinical indicators for pulmonary arterial hypertension in thalassemia. J Med Assoc Thai. 2012;95(1):16-21.

Clinical Indicators for Pulmonary Arterial Hypertension in Thalassemia

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Objective: *To explore clinical indicators for pulmonary arterial hypertension (PAH) in thalassemia (Thal).*
Material and Method: *A study was conducted in thalassemia patients at Chiang Rai Hospital, Chiang Rai, Thailand. Pulmonary artery systolic pressure (PASP) was determined by doppler echocardiography and PAH was defined as PASP > 35 mmHg. Patient characteristics were extracted from medical records. Characteristics of patients with and without PAH were compared. Risk indicators were explored with logistic regression analysis.*
Results: *Two hundred twenty four patients were included, 144 E/β-Thal, 37 homozygous β-Thal and 43 Hb H disease. There were 65 patients (29.0%) with PAH, 53 (81.5%) with E/β-Thal, 8 (12.3%) with homozygous β-Thal and 4 (6.2%) with Hb H disease. In a multivariable analysis, features significantly associated with PAH were E/β-Thal (OR = 1.98, 95% CI; 1.29-3.01) and post splenectomy status (OR = 2.36, 95% CI; 1.17-4.73).*
Conclusion: *Significant indicators for PAH in thalassemia were E/β-Thal and post splenectomy status.*

Keywords: *Thalassemia, Pulmonary Arterial hypertension, Splenectomy*

J Med Assoc Thai 2012; 95 (1): 16-21
Full text. e-Journal: *http://www.jmat.mat.or.th*

Pulmonary arterial hypertension (PAH) has been reported as one of the common cardiac complications in b-thalassemia (β-Thal) patients[1,2]. Sonakul et al reported thrombi in small pulmonary arteries in 44% of splenectomized hemoglobin E/β thalassemia (E/β-Thal) under autopsy[3]. In Greece, 10% of PAH was reported in thalassemia major, and more than 50% in thalassemia intermedia[2]. Its prevalence in Thailand was 43%[4]. Contributing factors of PAH are increased cardiac output from chronic anemia, increased pulmonary capillary wedge pressure likely from LV diastolic dysfunction from chronic iron overload and increased pulmonary vascular resistance from thrombotic pulmonary arteriopathy[5,6]. Early detection and prevention of severe PAH are effective and viable ways to decrease morbidities and mortalities. Few studies identified predictive characteristics of high pulmonary artery systolic pressure in thalassemia patients. PAH could not be detected early by clinical examination or electrocardiography or chest radiograph. Right heart catheterization is invasive, costly and has limited use in only large cardiac centers. Doppler echocardiography (ECHO) is more sensitive and is a commonly used noninvasive tool to detect PAH[7-9]. The authors studied PAH in thalassemia using ECHO to estimate the prevalence of pulmonary artery systolic pressure and to explore important clinical indicators for PAH in these patients.

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Material and Method

Two hundred twenty four adult (≥ 15 years) patients with thalassemia disease, who were treated at the hematology outpatient clinic, Chiang Rai Hospital, were evaluated and followed between January 2005 and June 2010, were included in this study. All patients had the diagnosis of Hb H disease, homozygous-Thal and E/β-Thal established by Hemoglobin (Hb) analysis

utilizing high performance liquid chromatography (HPLC). None of the patients had clinical evidence of other secondary causes of PAH, including HIV infection, collagen vascular diseases and chronic obstructive airway diseases. Acquired heart disease associated with pulmonary venous hypertension; mitral valve disease, congenital heart disease and hyperthyroidism were also excluded. No patients used the following classification of drugs: antiplatelet, anticoagulants, calcium channel blockers or vasodilators. The functional class status was defined by the New York Heart Association (NYHA) Classification (Class I: patients with no limitation of activities; they suffer no symptoms from ordinary activities, Class II: patients with slight, mild limitation of activity; they are comfortable with rest or with mild exertion, Class III: patients with marked limitation of activity; they are comfortable only at rest, Class IV: patients who should be at complete rest, confined to bed or chair; any physical activity brings on discomfort and symptoms occur at rest.). The investigation included history taking and clinical examination. Hematologic evaluation consisted of complete blood count, Hb analysis (HPLC), reticulocyte count, serum ferritin and creatinine level, liver function test, hepatitis profile, HIV screening and thyroid function test. Cardiac evaluation consisted of chest radiograph, electrocardiography (ECG) and ECHO. Pulmonary artery systolic pressure (PASP), estimated by ECHO, was performed on all patients. ECHO was done by the same cardiologist. The patients' history including type of thalassemia and splenectomy status were hidden during echocardiographic examinations. The present study protocol was approved by the institutional research ethics committee and was carried out in accordance with the Declaration of Helsinki.

Echocardiographic procedure

Complete two-dimensional, M mode and Doppler (pulsed wave, continuous wave and color) echocardiography was performed at rest with a (echo machine) using standard protocol following the American Society of Echocardiography guidelines. In a recruited patient, a tricuspid regurgitation jet was sought from all available mid-precordial and apical positions until a flow signal with the maximum spectral representation of the highest velocities could be obtained. Peak velocity was recorded from a holosystolic regurgitant jet. From the maximum velocity (V) of the regurgitant jet, the systolic pressure gradient (DP) between the right ventricle and right atrium was calculated by the modified Bernoulli equation ($DP = 4V^2$). Right atrial pressure was estimated by the response of the inferior vena cava diameter to inspiration. Right atrial pressure was assumed to be 5 mm Hg if the inferior vena cava completely collapsed with inspiration, 10 mmHg if the inferior vena cava diameter decreased more than 50% during inspiration, and 15 mmHg if it decreased less than 50%. If the inferior vena cavas diameter was larger than 2.5 cm and reduced by less than 50% during inspiration, right atrial pressure was assumed to be 20 mmHg. Adding the transtricuspid gradient to the mean right atrial pressure provided the right ventricular systolic pressure or peak systolic pulmonary arterial pressure in the absence of right ventricular outflow tract obstruction. Pulmonary hypertension was defined as a PASP greater than 35 mmHg[9]. In case absent of TR, the end diastolic pulmonic regurgitant (PR) velocity was calculated using the pressure gradient between the pulmonary artery and right ventricular end diastolic pressure. Pulmonary artery pressure = $4 (VPR)^2$ + right atrial pressure[10].

Statistical analysis

Descriptive statistics were used. Baseline characteristics were compared using the Chi-squared or Fisher's exact test for categorical variables, t-test or Mann-Whitney U-test or Wilcoxon ranksum test was used to compare the mean difference of continuous variables where appropriated. Univariable and multivariable logistic regression analysis were used to identify factors associated with PAH. This data was presented by frequency, percentage, mean, median, standard deviation (SD), inter-quartile range (IQR), Odds ratio, 95% confidence interval and p-value. A p-value of less than 0.05 was considered as statistically significant.

Results

Patient characteristics of 224 thalassemia patients are summarized in Table 1. 144 E/β-Thal, 37 homozygous β-Thal and 43 Hb H disease were evaluated. PAH was detected in 65 (29.02%) patients, 53 (81.54%) E/β-Thal, 8 (12.31%) homozygous β-Thal and 4 (6.15%) Hb H disease. The mean age was 35.37 $\pm$ 15.67 years. The mean PASP was 50.66 $\pm$ 13.53 mm Hg. All patients had normal left ventricular ejection fraction. The PAH group had a higher proportion than that of the non-PAH group for E/β-thal (53 (81.54) vs. 91 (57.23), p = 0.001), splenectomy (44 (67.69%) vs. 71 (44.65%), p = 0.002), the mean duration after splenectomy (19.75 $\pm$ 8.86 vs. 13.2 $\pm$ 6.38 years, p <

Table 1. Patient characteristics (n = 224)

Characteristics	PAH*, n (%)	Non-PAH, n (%)	p-value
Number of patients	65 (29.02)	159 (70.98)	-
Gender			0.471
Male	24 (36.92)	67 (42.14)	
Female	41 (63.08)	92 (57.86)	
Age (years)	35.37 ± 15.67	31.13 ± 15.76	0.069
Weight (kg)	43.32 ± 15.89	43.98 ± 9.16	0.694
Height (cm)	150.52 ± 8.21	153.21 ± 10.96	0.076
Type of thalassemia			0.001
Hb H disease	4 (6.15)	39 (24.53)	
Homozygous β-thal	8 (12.31)	29 (18.24)	
E/β-thal	53 (81.54)	91 (57.23)	
Splenectomy	44 (67.69)	71 (44.65)	0.002
Post splenectomy duration (years)	19.75 ± 8.86	13.2 ± 6.38	<0.001
Red cell transfusion (units/year) Median (IQR)	4 (11)	2 (8)	0.013
Endocrine complication	25 (38.46)	41 (25.79)	0.059
Functional class statusNYHA class			
I	8 (12.31)	58 (36.48)	
II	51 (78.46)	101 (63.52)	
III	6 (9.23)	0	<0.001
Clinical right heart failure**	9 (13.85)	0	<0.001
Systolic blood pressure (mmHg)	104.31 ± 13.45	106.99 ± 13.38	0.176
Diastolic blood pressure (mmHg)	62.28 ± 7.79	63.25 ± 9.12	0.454
Pulse rate (/min)	85.20 ± 10.84	86.66 ± 11.16	0.371
Oxygen saturation (%)	96.51 ± 2.02	97.85 ± 2.60	<0.001
Chest radiograph: suggestive of PAH***	43 (66.15)	10 (6.41)	<0.001
ECG suggestive of PAH****	33 (50.77)	3 (1.94)	<0.001
Hemoglobin (g/dl)	6.44 ± 1.36	6.84 ± 1.29	0.045
Corrected white blood cells (x 10^3/mL)	10.69 ± 5.41	10.92 ± 5.96	0.794
Platelets (x 10^3/mL)	393.92 ± 296.14	430.42 ± 296.93	0.259
Reticulocyte count (%)	7.46 ± 6.34	8.89 ± 6.86	0.298
Nucleated RBC/100 WBC, median (IQR)	28 (194)	11 (213)	0.334
Serum ferritin (mg/L)	3,759.54 ± 3267.67	2,307.66 ± 1886.46	<0.001
Indirect bilirubin (mg/dl)	2.0 ± 1.16	2.41 ± 1.94	0.063
HBsAg positive	2 (3.17)	9 (5.77)	0.426
Anti-HCV positive	8 (12.70)	18 (11.54)	0.810
Echocardiographic findings			
PASP (mmHg)	50.66 ± 13.53	26.05 ± 5.89	-
36-50 mmHg (mild)	40 (61.54)	-	
51-70 mmHg (moderate)	17 (26.15)	-	
> 70 mmHg (severe)	8 (12.31)	-	
LVEF (%)	62.17 ± 7.22	63.25 ± 7.02	0.301
LVESd (mm)	9.43 ± 2.05	9.06 ± 1.84	0.196
LVEDd (mm)	51.55 ± 6.42	50.08 ± 5.52	0.089
MPAd (mm)	26.61 ± 5.19	24.16 ± 3.33	0.005
RVd (mm)	28.55 ± 5.69	27.08 ± 4.34	0.084
Poor right ventricular systolic function	7 (10.77)	0	<0.001
Tricuspid regurgitation	61 (93.85)	32 (20.25)	<0.001
Diastolic function			
Mitral valve E:A ratio	1.38 ± 0.26	1.43 ± 0.51	0.913
Mitral valve DT (ms)	204.57 ± 52.98	220.60 ± 46.61	0.441

* Pulmonary arterial hypertension
** Elevated jugular venous pressure, hepatojugular reflux and edema
*** right interlobar pulmonary diameter of grater > 16 mm and Hilar to thoracic ratio > 0.44
**** right-axis deviation, R/S ratio > 1 in lead V1-3, R/S ratio < 1 in lead V5 or V6, right atrial enlargement
DT = deceleration time; LVEDd = left ventricular end diastolic diameter; LVEF = left ventricular ejection fraction; LVESd = left ventricular end systolic diameter; MPAd = main pulmonary artery diameter; RVd = right ventricular diameter

0.001), functional class status (Fc II 51 (78.46%) vs. 101 (63.52%) and Fc III 6 (9.23%) vs. 0, $p < 0.001$), clinical right heart failure (9 (13.85%) vs. 0), $p < 0.001$), oxygen saturation (96.5 ± 2.02 vs. $97.85 \pm 2.60\%$, $p < 0.001$). Patients with PAH received more blood transfusions during the preceding 12 months than those without PAH (4 (11) vs. 2 (8) units, $p = 0.013$). The PAH group had a lower the hemoglobin concentration than the non-PAH group (6.44 ± 1.36 vs. 6.84 ± 1.29 g/dl), $p = 0.045$) and they had higher serum ferritin levels ($3,759.54 \pm 3,267.67$ vs. $2,307.66 \pm 1,886.46$ mg/L, $p < 0.001$).

There were no statistically significant differences in gender, age, height, weight, endocrine complications, blood pressure, pulse rate, white blood cell count, platelet counts, percentage of nucleated red blood cells (nRBCs) to white blood cell count (WBC), reticulocyte count and indirect bilirubin level between PAH and non-PAH group. All had normal creatinine levels. None of the patients had clinical evidence of thromboembolism. In the PAH group, only 43 of 65 chest radiographs (66.15%) and 33 of 65 ECGs (50.77%) showed evidence of PAH.

Echocardiographic findings showed that patients with PAH had a significantly larger main pulmonary artery diameter, and a higher percentage of patients with poor right ventricular systolic function and tricuspid regurgitation (26.61 ± 5.19 vs. 24.16 ± 3.33 mm, $p = 0.005$, 7 (10.77) vs. 0, $p < 0.001$ and 61 (93.85) vs. 32 (20.25), $p < 0.001$, respectively).

Univariable and multivariable analysis are shown in Table 2 and 3. In a multivariable analysis, features significantly associated with PAH were E/β-Thal (OR 1.98 [1.29-3.01]) and post splenectomy status (OR 2.36 [1.17-4.73]).

Discussion

The prevalence of PAH reported in the patients was 29%. None of them had clinical evidence of acute or chronic deep vein thrombosis (DVT) (unilateral leg swelling, dilated superficial veins, chronic leg ulcer) which correlated with the findings of Sonakul et al which did not report emboli from DVT in autopsy[3]. Regular blood transfusions and iron chelation had been reported to prevent PAH in thalassemia major[11], but the authors did not find that the patients without

Table 2. Univariable association between clinical indicators and PAH

Indicators	Odds ratio	95% CI	p-value
Type of thalassemia			
Hb H disease or Homozygous β-thal	1	-	-
E/β-thal	3.30	1.60-6.79	<0.001
Splenectomy	2.59	1.39-4.84	0.002
Post-splenectomy duration ≥ 15 years	4.68	1.95-11.19	<0.001
Number of red cell transfusion > 6 units/year	1.92	1.04-3.52	0.033
Hemoglobin ≤ 6 g/dl	2.15	1.16-3.96	0.012
Serum ferritin > 1,000 μg/L	3.79	1.48-9.70	0.003

Table 3. Multivariable association between clinical indicators and PAH

Indicators	Adjusted odds ratio	95% CI	p-value
Type of thalassemia			
Hb H disease or Homozygous β-thal	1	-	-
E/β-thal	1.98	1.29-3.01	0.002
Splenectomy*	2.36	1.17-4.73	0.016
Number of red cell transfusion > 6 units/year	1.69	0.80-3.56	0.166
Hemoglobin ≤ 6 g/dl	1.15	0.56-2.36	0.695
Serum ferritin > 1,000 μg/L	2.45	0.92-6.54	0.073

* Splenectomy and post-splenectomy duration are collinearity

PAH received more blood transfusions than the patients with PAH because of the number of unit blood transfusions was calculated for only the preceding 12 months. This higher level of blood transfusions may imply more severe disease in patients with PAH. The previous study showed that high serum ferritin level was significantly associated with pulmonary hypertension[12]. The authors also found 2.45 times the chance to detect PAH in the patients with serum ferritin higher than 1,000 μg/L. However, the association was not statistically significant. In the present study the authors propose that the predictive factors for PAH in thalassemia include, E/β-Thal, post splenectomy status, number of red cell transfusion, hemoglobin and serum ferritin level, which were 72.15% accurate.

In the authors' study, features significantly associated with PAH were E/β-Thal and post splenectomy status. These findings are consistent with the proposed pathogenesis of other authors. The possible mechanisms leading to PAH after splenectomy may involve nucleated red blood cells, platelet activation and the coagulation cascade[5,6,13-17]. Moreover, thalassemic red blood cells can induce thrombotic complications. These cells have increased membrane expression of anionic phospholipids that accelerate thrombin generation and activate platelets[14]. The mechanisms are likely to be intensified in splenectomized patients with thalassemia, as they have more abnormal red blood cells and red blood cell precursors than their non-splenectomized counterparts. Pathophysiologic changes in post-splenectomy β-Thal leading to PAH are increased circulating PS (phosphatidylserine)-exposed RBCs which facilitate the coagulation process and activated platelets which cause vasculopathy and microthrombi leading to increased pulmonary vascular resistance index and vascular occlusion[5,6].

PAH can be associated with all kinds of chronic hemolytic anemia[14,18]. Therapeutic strategies of PAH are still controversial. Some authors suggested that the development of PAH could be prevented, by starting transfusion and chelation therapy early in life for patients with thalassemia intermedia[2].

Conclusion

PAH is the main feature of cardiac complications in β-Thal. The authors' findings indicate that the indicators for PAH are E/β-Thal and post splenectomy status. A serial ECHO to estimate PASP in high-risk groups for early appropriate treatment may be beneficial. Furthermore, the authors' studies evaluated the role of antiplatelets and regular blood transfusions in thalassaemia with PAH.

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Potential conflicts of interest

None.

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ลักษณะบ่งชี้ภาวะความดันในหลอดเลือดแดงปอดสูงในผู้ป่วยธาลัสซีเมีย

นลวันท์ เชื้อเมืองพาน, วัฒนา วงศ์เทพเตียน, วีระศักดิ์ นาวารวงศ์, อภิชาต สุคนธสรรพ์, สุภร จันท์จารุณี, ชไมพร ทวิชศรี, ชยันตร์ธร ปทุมานนท์

วัตถุประสงค์: *เพื่อศึกษาลักษณะบ่งชี้ภาวะความดันในหลอดเลือดแดงปอดสูงในผู้ป่วยธาลัสซีเมีย*
วัสดุและวิธีการ: *เป็นการศึกษาในผู้ป่วยธาลัสซีเมียที่โรงพยาบาลเชียงรายประชานุเคราะห์โดยใช้ Doppler echocardiography ในการวัดค่า pulmonary artery systolic pressure (PASP) โดย PASP > 35 mmHg ถือว่ามีภาวะความดันในหลอดเลือดแดงปอดสูง และวิเคราะห์เปรียบเทียบปัจจัยที่แตกต่างระหว่างกลุ่มที่มีและไม่มีภาวะดังกล่าว*
ผลการศึกษา: *ผู้ป่วย 224 ราย: E/β-Thal 144 ราย homozygous β-Thal 37 ราย และ Hb H disease 43 ราย พบภาวะความดันในหลอดเลือดแดงปอดสูงทั้งหมด 65 ราย (29.02%) เป็น E/β-Thal 53 ราย (81.54%) homozygous β-Thal 8 ราย (12.31%) Hb H disease 4 ราย (6.15%) จากการวิเคราะห์ถดถอยลอจิสติกพบลักษณะที่สัมพันธ์กับภาวะความดันในหลอดเลือดแดงปอดสูงคือโรค E/β-Thal (OR 1.98 [1.29-3.01]) และภาวะหลังตัดม้าม (OR 2.36 [1.17-4.73])*
สรุป: *ลักษณะบ่งชี้ภาวะความดันในหลอดเลือดแดงปอดสูงในผู้ป่วยธาลัสซีเมียคือ E/β-Thal และภาวะหลังตัดม้าม*

Appendix C

Chueamuangphan N, Wongtheptian W, Patumanond J, Sukornthasarn A, Chuncharunee S, Tawichasri C, Nawarawong W. Effect of acetylsalicylic acid on thalassemia with pulmonary arterial hypertension. Int J Gen Med. 2014;7:525-9

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ORIGINAL RESEARCH

Effect of acetylsalicylic acid on thalassemia with pulmonary arterial hypertension

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Objective: To compare pulmonary artery systolic pressure (PASP) between thalassemic patients with pulmonary arterial hypertension (PAH) for whom acetylsalicylic acid (ASA) was and was not prescribed after 1 year.

Methods: A retrospective cohort study was conducted at the hematological outpatient clinic at Chiang Rai Hospital, Chiang Rai, Thailand. All new cases of thalassemia with PAH from January 2007 to January 2012 were studied at the first month and at 12 months. The patients were classified into two groups. In one group, ASA 81 mg daily was prescribed for 1 year, whereas in another group no ASA was prescribed, due to its contraindications, which included bleeding, gastrointestinal side effects, and thrombocytopenia. PASP, estimated by a Doppler echocardiography, was measured by the same cardiologist. Propensity score adjustment was used to control confounding variables by indication and contraindication. Multivariable regression analysis was used to evaluate the effects of ASA.

Results: Of the 63 thalassemia patients with PAH, there were 47 (74.6%) in the ASA group and 16 (25.4%) in the no ASA group. ASA, as compared with no ASA, did not significantly reduce PASP (adjusted difference −0.95; 95% confidence interval −16.99 to 15.10; P=0.906).

Conclusion: Low-dose ASA may not have a beneficial effect on PASP after 1 year of treatment of PAH in thalassemia.

Keywords: thalassemia, pulmonary arterial hypertension, acetylsalicylic acid

Introduction

Pulmonary arterial hypertension (PAH) is a cardiovascular complication that causes death in thalassemic patients. PAH in thalassemia was reported with a high incidence in several studies.[1–4] There was evidence that PAH in thalassemia is associated with platelet activation.[5–7] Acetylsalicylic acid (ASA) lowers the thromboxane–prostaglandin I_2 (Tx–PGI_2) ratio in PAH and inhibits platelet activity.[8] A previous cohort study found that the rise of arterial partial pressure of oxygen (PaO_2) in ten out of the 12 β-thalassemic patients after ASA ten grains or persantin administration for 2–4 weeks indicates that the observed hypoxemia is due to reversible platelet aggregation.[9] A recent study showed that ASA decreased pulmonary artery pressure, reduced right ventricular hypertrophy, and improved survival in the monocrotaline animal model of PAH.[10] The erythrocytes and platelets of thalassemic patients contained higher levels of reactive oxygen and lower levels of intracellular glutathione than normal erythrocytes and platelets[11] that play a role in oxidative stress, thereby leading to the proper therapy.

The standard medication for PAH in thalassemia is not well understood. The mechanism of action of the drugs for PAH is not known. Studies of antiplatelet therapy

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are required before there are conclusive treatment guidelines for thalassemic patients with PAH. In clinical practice, ASA has been used to prevent and treat PAH in thalassemia in Thailand. There were few studies of ASA or antiplatelet therapy in these cases. The aim of the research was to compare after 1 year of follow-up the pulmonary artery systolic pressure (PASP) of patients with thalassemia and PAH to whom ASA was and was not prescribed.

Materials and methods

A retrospective cohort study was conducted at the hematological outpatient clinic at Chiang Rai Hospital, Chiang Rai, Thailand. All new adult (aged ≥15 years) cases of thalassemia with PAH defined as PASP >35 mmHg by Doppler echocardiography (echo) from January 2007 to January 2012 were evaluated and followed from the first month to 12 months later. The patients were classified into two groups. In one group, ASA 81 mg was prescribed daily for 1 year, and in another group, no ASA was prescribed, due to contraindications for ASA (including bleeding, gastrointestinal side effects, and thrombocytopenia [platelets $<100\times10^3/\mu L$]). The physician evaluated drug compliance and side effects. PASP, functional class status, clinical right heart failure, and oxygen saturation were measured at baseline and at the end of 12 months. The functional class status was defined by the New York Heart Association (NYHA) classification.[12] Patients with other causes of PAH or who used other antiplatelets and/or anticoagulants were excluded. The study protocol was approved by the Institutional Research Ethics Committee and was carried out in accordance with the Declaration of Helsinki.

Echo procedure

Echo was done by the same cardiologist and the patients' history was blinded. Complete two-dimensional, M-mode, and Doppler (pulsed wave, continuous wave, and color) echo was performed at rest. In the study patients, a tricuspid regurgitation jet was sought from all available midprecordial and apical positions until a flow signal with the maximum spectral representation of the highest velocities was obtained. Peak velocity was recorded from a holosystolic regurgitant jet. From the maximum velocity (V) of the regurgitant jet, the systolic pressure gradient (ΔP) between the right ventricle and the right atrium was calculated by modified Bernoulli equation ($\Delta P = 4V^2$).[13] Right atrial pressure was estimated by the response of the inferior vena cava diameter to inspiration. Right atrial pressure was assumed to be 5 mmHg if the inferior vena cava completely collapsed with inspiration, 10 mmHg if the inferior vena cava diameter decreased >50% during inspiration, and 15 mmHg if it decreased <50%. If the inferior vena cava diameter was >2.5 cm and reduced by <50% during inspiration, right atrial pressure was assumed to be 20 mmHg.[14] Adding the transtricuspid gradient to the mean right atrial pressure provided the right ventricular systolic pressure or peak systolic pulmonary arterial pressure in the absence of right ventricular outflow tract obstruction. PAH was defined as PASP >35 mmHg.[15] In cases with an absence of tricuspid regurgitation, at the end of the diastolic pulmonic regurgitant the velocity was calculated using the pressure gradient between the pulmonary artery and right ventricular end diastolic pressure: pulmonary artery pressure =4 $(VPR)^2$ + right atrial pressure.

Statistical analysis

The baseline characteristics were compared using exact probability tests for categorical variables; Student's *t*-test or Wilcoxon rank-sum test was used to compare the mean difference of continuous variables. Propensity score adjustment was used to control confounding by ASA indication and contraindication. Propensity scores for prescribing ASA versus no ASA were calculated from a logistic regression model that estimated the likelihood of prescribing ASA based on the observed patient characteristics. Gaussian and an exponential risk regression were carried out. Univariable and multivariable regression analyses were used to evaluate the effects of ASA. Data are presented by frequency, percentage, mean, standard deviation (SD), beta coefficient, 95% confidence interval [CI], and *P*-value. All statistical analyses were two-tailed. A *P*-value of <0.05 was considered statistically significant.

Results

A total of 63 thalassemia patients with PAH, 53 with E/β-thal, six with homozygous β-thal, and four with Hb H disease were evaluated. There were 47 (74.6%) in the ASA group and 16 (25.4%) in the no ASA group. Patients in the second group had the following contraindications for ASA: seven (43.7%) bleeding, seven (43.7%) gastrointestinal side effects, and two (12.5%) thrombocytopenia. The mean age was 35.9±16.8 years and 28.3±14.1 years, and the mean PASP was 51.9±13.7 mmHg and 45.6±9.9 mmHg in the ASA group and the no ASA group. The baseline characteristics of the patients did not differ significantly except for red cell transfusion and right ventricular diameter (Table 1). The patients in the ASA group received fewer blood transfusions during the follow-up time of 12 months than those without ASA (6.2±4.8 vs 11.0±4.6 units, *P*=0.002), and the ASA group

Table 1 Baseline characteristics of the patients[a]

Characteristic	ASA, n=47	No ASA, n=16	*P*-value
Male (n [%])	16 (34.0)	6 (37.5)	0.515
Age (years)	35.9±16.8	28.3±14.1	0.112
Weight (kg)	42.3±6.2	39.0±8.3	0.104
Height (cm)	150.6±8.2	147.0±12.1	0.192
Type of thalassemia (n [%])			
Hemoglobin E/β-thalassemia	41 (87.2)	12 (75.0)	0.288
Homozygous β-thalassemia	3 (6.4)	3 (18.7)	
Hemoglobin H disease	3 (6.4)	1 (6.3)	
Splenectomy (n [%])	29 (61.7)	13 (81.2)	0.129
Red cell transfusion (units/year)	6.2±4.8	11±4.6	0.002
Transfusion dependent (≥9 units/year) (n [%])	14 (29.8)	12 (75.0)	0.003
Nontransfusion dependent	33 (70.2)	4 (25.0)	
Functional class status: NYHA (n [%])			
I	5 (10.6)	0	0.463
II	38 (80.9)	14 (87.5)	
III	4 (8.5)	2 (12.5)	
Clinical right heart failure* (n [%])	8 (17.0)	1 (6.3)	0.270
Systolic blood pressure (mmHg)	106.9±15.0	102.0±9.2	0.218
Diastolic blood pressure (mmHg)	62.6±8.0	60.5±6.8	0.352
Pulse rate (/minute)	85.0±10.4	90.8±13.1	0.078
Oxygen saturation (%)	95.9±2.1	96.4±1.6	0.374
Hemoglobin (g/dL)	6.3±0.9	6.3±0.8	0.903
Corrected WBCs ($\times 10^3/\mu L$)	11.1±5.8	14.4±5.7	0.054
Platelets ($\times 10^3/\mu L$)	369.3±28.7	501.4±27.5	0.114
Nucleated RBCs/100 WBCs	192.1±248.4	117.5±91.0	0.888
PT (seconds)	13.1±1.2	13.4±1.0	0.394
PTT (seconds)	31.2±3.1	30.7±3.1	0.560
INR	1.2±0.1	1.2±0.0	0.339
Aspartate transaminase (IU/L)	66.5±48.0	72.0±48.2	0.540
Alanine transaminase (IU/L)	51±35	52±37	0.993
Total bilirubin (mg/dL)	2.7±1.3	2.6±1.2	0.781
HBsAg positive (n [%])	2 (4.2)	0	0.554
Anti-HCV positive (n [%])	5 (10.6)	4 (25)	0.157
Serum creatinine (mg/dL)	0.6±0.1	0.71±0.2	0.165
Serum ferritin (μg/L)	3,006±2797	3,345±1510	0.067
Echocardiographic findings			
PASP (mmHg)	51.9±13.7	45.6±9.9	0.096
PASP (mmHg) severity (n [%])			
36–50 mmHg (mild)	25 (53.2)	12 (75.0)	0.404
51–70 mmHg (moderate)	15 (31.9)	3 (18.7)	
>70 mmHg (severe)	7 (14.9)	1 (6.3)	
LVEF (%)	62.7±7.8	63.5±7.4	0.718
LVESd (mm)	9.5±2.1	9.0±2.2	0.445
LVEDd (mm)	51.5±6.0	49.4±7.6	0.256
MPAd (mm)	25.9±4.2	25.0±3.0	0.464
RVd (mm)	29.5±5.8	25.5±4.2	0.016
Poor right ventricular systolic function (n [%])	7 (14.8)	0	0.114
Diastolic function, n (%)			
Mitral valve E:A ratio	1.4±0.4	1.2±0.2	0.515
Mitral valve DT (ms)	199±35	202±88	0.941
Propensity score	0.8±0.2	0.2±0.2	<0.001

Notes: [a]Values shown are mean ± standard deviation unless otherwise specified; *elevated jugular venous pressure, hepatojugular reflux, and edema.

Abbreviations: ASA, acetylsalicylic acid; DT, deceleration time; E:A, ratio of the early (E) to late (A) ventricular filling velocities; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; INR, international normalized ratio; LVEDd, left ventricular end diastolic diameter; LVEF, left ventricular ejection fraction; LVESd, left ventricular end systolic diameter; MPAd, main pulmonary artery diameter; NYHA, New York Heart Association; PASP, pulmonary artery systolic pressure; PT, prothrombin time; PTT, partial thromboplastin time; RBCs, red blood cells; RVd, right ventricular diameter; WBCs, white blood cells.

had wider right ventricular diameter than the no ASA group (29.5±5.8 vs 25.5±4.2 mm, *P*=0.016).

During the follow-up period of 12 months, PASP increased in 12 of 47 (25.5%) patients in the ASA group and in three of 16 (18.7%) in the no ASA group. Echo findings showed that the patients in the ASA group had a higher mean PASP than the no ASA group, but there was no statistical difference.

After adjusting for propensity score (based on type of thalassemia, splenectomy, O_2 saturation, NYHA classification, red cell transfusion, clinical right heart failure, hemoglobin level, platelet count, nucleated red cell, serum ferritin level, baseline PASP, left ventricular ejection fraction, main pulmonary artery diameter, right ventricular diameter, right ventricular systolic function, and diastolic function), there were no statistically significant differences in the functional class status, clinical right heart failure, and oxygen saturation between the two groups (Table 3). ASA, as compared with no ASA, did not significantly reduce PASP (adjusted difference –0.95; 95% CI –16.99 to 15.10; *P*=0.906). None of the patients had deep vein thrombosis, ischemic stroke and pulmonary embolism.

Discussion

In the present study, ASA did not improve the functional class status, clinical right heart failure, and oxygen saturation and did not have a significant effect on PASP after 12 months. Both the ASA and the no ASA groups had reduced PASP

Table 2 Clinical outcomes and echocardiographic findings at 12 months[a]

Outcome	ASA, n=47	No ASA, n=16	*P*-value
Functional class status: NYHA (n [%])			
I	5 (10.6)	1 (6.3)	0.841
II	39 (83.0)	15 (93.7)	
III	3 (6.4)	0	
Clinical right heart failure (n [%])	6 (12.7)	1 (6.3)	0.424
O_2 saturation (%)	96.7±2.2	97.1±1.7	0.492
Echocardiographic findings			
PASP (mmHg)	47.2±16.9	41.2±11.6	0.197
LVEF (%)	64.9±7.5	64.1±6.4	0.700
LVESd (mm)	9.6±2.0	9.8±2.6	0.759
LVEDd (mm)	50.6±5.6	48.7±6.9	0.266
MPAd (mm)	25.8±4.3	24.5±3.3	0.249
RVd (mm)	29.7±6.2	26.6±3.0	0.057
Poor right ventricular systolic function (n [%])	6 (12.7)	0	0.158
Diastolic function			
Mitral valve E:A ratio	1.5±0.5	1.7±0.9	0.395
Mitral valve DT (ms)	205±28	217±29	0.382

Note: [a]Values shown are mean ± standard deviation unless otherwise specified.
Abbreviations: ASA, acetylsalicylic acid; DT, deceleration time; E:A, ratio of the early (E) to late (A) ventricular filling velocities; LVEDd, left ventricular end diastolic diameter; LVEF, left ventricular ejection fraction; LVESd, left ventricular end systolic diameter; MPAd, main pulmonary artery diameter; NYHA, New York Heart Association; PASP, pulmonary artery systolic pressure; RVd, right ventricular diameter.

Table 3 Effect of acetylsalicylic acid on clinical outcomes and echocardiographic findings

Outcome[a]	Adjusted difference* (95% CI)	*P*-value
Functional class status: NYHA class (n [%])[b]	0.30 (−3.18 to 3.78)	0.865
Clinical right heart failure (n [%])[c]	−0.001 (−3.86 to 3.86)	0.999
O_2 saturation (%)	0.31 (−1.71 to 2.33)	0.757
Echocardiographic findings		
PASP (mmHg)	−0.95 (−16.99 to 15.10)	0.906
LVEF (%)	−0.66 (−9.24 to 7.92)	0.877
LVESd (mm)[d]	−0.76 (−4.08 to 2.56)	0.655
LVEDd (mm)	−1.47 (−8.50 to 5.56)	0.673
MPAd (mm)	1.19 (−2.67 to 5.06)	0.534
RVd (mm)[d]	0.83 (−4.62 to 6.29)	0.765
Poor right ventricular systolic function, n (%)[c]	15.37 (−2,219 to 2,250)	0.989
Diastolic function		
Mitral valve E:A ratio[d]	0.46 (−1.78 to 2.70)	0.687
Mitral valve DT (ms)[d]	−25.62 (−47.89 to −3.36)	0.024

Notes: *Adjusted for propensity score (type of thalassemia, splenectomy, O_2 saturation, NYHA classification, red cell transfusion, clinical right heart failure, hemoglobin level, platelet count, nucleated red cell, serum ferritin level, baseline PASP, LVEF, MPAd, RVd, right ventricular systolic function, and diastolic function). [a]Gaussian regression unless otherwise indicated; [b]ordered logistic regression; [c]generalized linear models: extensions to the binomial family; [d]generalized linear models: Poisson regression.
Abbreviations: CI, confidence interval; DT, deceleration time; E:A, ratio of the early (E) to late (A) ventricular filling velocities; LVEF, left ventricular ejection fraction; MPAd, main pulmonary artery diameter; NYHA, New York Heart Association; PASP, pulmonary artery systolic pressure; RVd, right ventricular diameter.

by −4.7±11.0 mmHg and −4.3±7.1 mmHg with no clinical or statistical significance. The results of this study were similar to those of a randomized clinical trial of ASA and simvastatin for PAH, which concluded that the results did not support the routine treatment of patients with PAH with these medications.[16] And thalassemia patients who experienced a thromboembolic event and received ASA afterwards had a lower recurrence of thromboembolic event compared with those who were not taking ASA, although these differences were not statistically significant.[17] Subgroup analysis of E/β-thal; ASA, as compared with no ASA, did not significantly reduce the PASP (adjusted difference −3.62; 95% CI −11.84 to 4.60; *P*=0.225). For the transfusion- and nontransfusion-dependent patients, ASA did not significantly change the PASP.

In contrast to the previous study that reported that there was a rise of arterial PaO_2 after high-dose ASA administration in thalassemia patients with PAH,[9] we found that oxygen saturation rose in the patients who had received low-dose ASA but without statistical significance (adjusted difference 0.31; 95% CI −1.71 to 2.33; *P*=0.757).

PAH developed in thalassemic patients because of platelet activation and hypercoagulable state.[18,19] Therefore, it was reasonable that antiplatelet therapy alone may be less effective for PAH in thalassemia. Its role in the prevention of PAH in thalassemia should be evaluated.

Further studies of PAH management will be required before conclusive recommendations can be made for antiplatelet or anticoagulant therapy or prevention for high risk of PAH in thalassemia (postsplenectomy status[3]).

The present study may have some limitations, as it was not a randomized controlled trial, the duration of treatment was only 12 months, and the 6-minute walk test was not used as a clinical outcome due to its retrospective nature. The lack of cardiac catheterization to confirm PASP is a limitation for a therapeutic trial of PAH.[20] However, echo is more sensitive and is a commonly used noninvasive tool to screen PAH. More evidence from observational studies or preferably randomized clinical trials may be required before it can be concluded that ASA would offer any beneficial effects to these patients.

The current therapies for PAH in thalassemia include the use of sildenafil[21,22] and bosentan.[23] However, a large cohort of patients on the topic are needed before definite recommendations can be made.

Conclusion

The present findings suggested that low-dose ASA may not have a beneficial effect on PASP after 1-year treatment of PAH in thalassemia.

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Disclosure

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ORIGINAL RESEARCH

Benefits of chronic blood transfusion in hemoglobin E/β thalassemia with pulmonary arterial hypertension

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Objective: The aim of the research reported here was to compare pulmonary artery systolic pressure (PASP) and 6-minute walk distance after 1 year of follow-up in hemoglobin E/β thalassemia (E/β-Thal) with pulmonary arterial hypertension (PAH) patients who received chronic blood transfusions versus those who received occasional transfusions.

Methods: A nonrandomized clinical trial was conducted at the Hematological Outpatient Clinic of Chiang Rai Hospital, Thailand. All adult cases of E/β-Thal with PAH (defined as PASP >35 mmHg by Doppler echocardiography) were evaluated and followed for the next 12 months. The patients were classified into two groups by patient preference. Group 1 patients received chronic blood transfusions – one to two units of leukocyte-poor packed red cells every 2–4 weeks – over 1 year to maintain pre-transfusion hemoglobin levels of ≥7.0 g/dL. Group 2 patients received occasional transfusions over the course of 1 year, with more than 4 weeks between transfusions. All patients were treated with iron chelation when serum ferritin levels were ≥1,000 µg/dL. PASP and the 6-minute walk distance were evaluated at baseline and at 6 and 12 months. Propensity score adjustment was used to control for confounding by indication and contraindication. Multivariable regression analysis was used to evaluate the effects of chronic blood transfusion.

Results: There were 16 (53.3%) patients in Group 1 and 14 (46.7%) in Group 2. At 12 months, patients in Group 1 had a greater reduction in PASP than those in Group 1 (adjusted mean difference, −16.83; 95% confidence interval, −26.35 to −7.32; *P*=0.001). The 6-minute walk distance at 12 months in Group 1 patients was greater than that in Group 2 patients (adjusted mean difference, 46.55; 95% confidence interval, 18.08 to 75.02; *P*=0.001).

Conclusion: This study found evidence that chronic blood transfusions may have beneficial effects in PAH in thalassemia patients over 1 year.

Keywords: pulmonary artery systolic pressure, Thailand, 6-minute walk distance, leukocyte-poor packed red cells

Introduction

"Pulmonary arterial hypertension" (PAH) is a common cardiovascular complication in thalassemic patients. It is a progressive disease that causes exercise limitation, right ventricular failure, and death. One study found that almost 60% of cases in a large cohort of 110 thalassemia intermedia (TI) patients had developed PAH.[1] Its prevalence in Thailand has been found to be 43%.[2] Another study has reported thrombi in small pulmonary arteries in 44% of hemoglobin E/β thalassemia (E/β-Thal) autopsies.[3] PAH is due to increased pulmonary vascular resistance.[1] In thalassemia, PAH could be due to an increased pulmonary blood flow from chronic anemia, and the oxidative stress resulting from chronic hemolysis is enhanced by iron overload.

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The possible mechanisms leading to PAH in thalassemia may involve platelet activation and the coagulation cascade.[4–10] Therapeutic strategies for dealing with PAH in thalassemia are controversial. Some authors have suggested that the PAH development can be prevented by starting transfusion and chelation therapy early in life for patients with TI.[1,11]

In the study reported here, we compared pulmonary artery systolic pressure (PASP) and 6-minute walk distance after 1 year of follow-up in E/β-Thal with PAH patients who received chronic blood transfusions versus those who received occasional transfusions.

Materials and methods

A nonrandomized clinical trial was conducted at the Hematological Outpatient Clinic at Chiang Rai Hospital, Thailand. All adult (≥15 years) cases of E/β-Thal with PAH (defined as PASP >35 mmHg by Doppler echocardiography) attending the clinic between June 2011 and November 2012 were evaluated and followed for the next 12 months. Inclusion criteria consisted of TI and serum ferritin level <2,500 μg/dL in patients capable of being followed-up who signed informed consent to participate in the study. Exclusion criteria were patients who had clinical evidence of other secondary causes of PAH, including human immunodeficiency virus infection, collagen vascular diseases, cirrhosis, and chronic obstructive airway diseases, acquired heart disease associated with pulmonary venous hypertension; mitral valve disease; congenital heart disease; and hyperthyroidism.

After the physician advised patients that they may gain some benefit from chronic blood transfusions; patients were classified into one of two groups according to their preference. Group 1 patients were those with a preference for chronic blood transfusions. Patients in this group would receive one to two units of leukocyte-poor packed red cells every 2–4 weeks to maintain the pre-transfusion hemoglobin level of ≥7.0 g/dL over 1 year. Group 2 patients, comprising those patients who indicated a preference for occasional blood transfusions, would receive occasional transfusions not more often than every 4 weeks. All patients were treated with medications following standard guidelines, including intensive chelation therapy when their serum ferritin level was above 1,000 μg/dL.

Project exclusion rule

The patients who developed an alloantibody to red blood cells and for whom the blood bank could not regularly supply compatible blood and/or who had serious blood transfusion complications such as anaphylaxis, acute pulmonary edema, or a serum ferritin level >2,500 μg/dL (serum ferritin monitoring was undertaken every 3 months) were excluded from the trial and were managed by the hospital's standard guidelines.

Blood transfusion

All transfusions were undertaken following the standard operating procedures of our blood bank, which adhere to the Thai Red Cross Society's National Blood Center.[12] To prevent alloimmunization, transfusions were of blood phenotypically matched for Rhesus (Rh) and other alloantibodies, with leukocyte-poor (depleted) packed red cells at 250–350 cc/unit, and the standard complication monitoring system for blood transfusions was observed.

Indicators

PASP and 6-minute walk distance[13] were measured at baseline and at 6 and 12 months. The clinical outcomes consisted of functional class status, clinical right heart failure, and oxygen saturation. Functional class status was defined by the New York Heart Association (NYHA) Functional Classification[14] (I: no symptoms with ordinary physical activity; II: symptoms with ordinary activity and slight limitation of activity; III: symptoms with less than ordinary activity, marked limitation of activity; IV: symptoms with any activity or even at rest). Echocardiography was performed by the same cardiologist. The patients' history, including blood transfusion frequency and splenectomy status, was blinded during echocardiographic examinations.

The study protocol was approved by the Institutional Research Ethics Committee, Faculty of Medicine, Chiang Mai University and Chiang Rai Hospital.

Study size estimation

The sample size was estimated from a pilot study comparing the mean PASP difference between two independent groups. A mean PASP of the patients was recorded before and after treatments for 1 year. The calculation was based on a type I error at 0.05 and type II error at 0.1. When using a standard statistical package to calculate the number of samples needed with the ratio of the patients of the two groups being 1:1, six subjects per treatment group were required. We aimed to collect the data from at least 14 patients per treatment group.

Echocardiographic procedure

Complete two-dimensional, M-mode, and Doppler (pulsed wave, continuous wave, and color) echocardiography was performed at rest. In the study patients, a tricuspid regurgitation jet was sought from all available mid-precordial

and apical positions until a flow signal with the maximum spectral representation of the highest velocities was obtained. Peak velocity was recorded from a holosystolic regurgitant jet. From the maximum velocity (V) of the regurgitant jet, the systolic pressure gradient (ΔP) between the right ventricle and the right atrium was calculated by the modified Bernoulli equation ($\Delta P = 4V^2$).[15] Right atrial pressure was estimated by the response of the inferior vena cava diameter to inspiration. Right atrial pressure was assumed to be 5 mmHg if the inferior vena cava completely collapsed with inspiration, 10 mmHg if the inferior vena cava diameter decreased more than 50% during inspiration, and 15 mmHg if it decreased less than 50%. If the inferior vena cava diameter was larger than 2.5 cm and reduced by less than 50% during inspiration, right atrial pressure was assumed to be 20 mmHg.[16] Adding the transtricuspid gradient to the mean right atrial pressure provided the right ventricular systolic pressure or peak systolic pulmonary arterial pressure in the absence of right ventricular outflow tract obstruction. PAH was defined as a PASP >35 mmHg.[17] In cases with an absence of tricuspid regurgitation, the end of the diastolic pulmonic regurgitant flow velocity was calculated using the pressure gradient between the pulmonary artery and right ventricular end diastolic pressure. Pulmonary artery pressure $= 4 (VPR)^2 +$ right atrial pressure.

Statistical analysis

The baseline characteristics were compared using exact probability tests for categorical variables; Student's *t*-test or Wilcoxon rank-sum test was used to compare the mean difference of continuous variables. Propensity score adjustment was used to control confounding by chronic blood transfusion indication and contraindication. Propensity scores for receiving chronic blood transfusions versus occasional transfusions were calculated from a logistic regression model that estimated the likelihood of receiving chronic blood transfusions based on the observed patient characteristics. Gaussian and an exponential risk regression were carried out. Uni-variable and multivariable regression analyses were used to evaluate the effects of chronic blood transfusions. Data are presented by frequency, percentage, mean, median, standard deviation (SD), range, beta coefficient, 95% confidence interval, and *P*-value. All statistical analyses were two-tailed. A *P*-value of <0.05 was considered statistically significant.

Results

A total of 30 patients with E/β-Thal were enrolled in the study, 16 (53.3%) in the chronic blood transfusion group (Group 1) and 14 (46.7%) in the occasional transfusion group (Group 2). The mean age of the patients was 36.1±14.6 and 34.1±15.2 years. Patients' mean PASP was 59.7±15.1 and 49.0±13.8 mmHg, while eight (50.0%) and four (28.6%) patients were post-splenectomy in Group 1 and Group 2, respectively. The baseline characteristics of the patients did not differ significantly between the two groups, except in terms of NYHA classification and PAH severity (Table 1). More patients in Group 1 had a functional NYHA class of III than those in Group 2 (5 [31.3%] vs 0 [%] patients, *P*=0.045) and PAH severity was greater in Group 1 than in Group 2 (mild: 4 [25.0%] vs 10 [71.4%] patients, *P*=0.049; moderate: 8 [50.0%] vs 2 [14.3%]; severe: 4 [25.0%] vs 2 [14.3%]).

During the follow-up period, the mean PASP was 50.4±14.6 versus 49.4±16.9 at 6 months and 48.8±18.8 versus 54.1±21.4 mmHg at 12 months, in Groups 1 and 2, respectively. At 6 months, mean 6-minute walk distance was 388.7±59.2 versus 368.6±61.1 m, and 403.7±53.8 versus 350.0±82.9 m at 12 months in Group 1 and Group 2, respectively, without statistical significance (Table 2).

After adjusting for propensity score (age, splenectomy, acetyl salicylic acid, NYHA class, clinical right heart failure, O_2 saturation, baseline PASP, left ventricular ejection fraction, diastolic function, hemoglobin level, platelet count, nucleated red cell, baseline serum ferritin level, and baseline 6-minute walk distance) and right ventricular systolic function, after 12 months of treatment, Group 1 had a mean PASP reduction greater than that of Group 2 (adjusted mean difference, –16.83; 95% confidence interval [CI], –26.35 to –7.32; *P*=0.001). The mean 6-minute walk distance at 12 months in Group 1 was greater than that in Group 2 (adjusted mean difference, 46.55; 95% CI, 18.08 to 75.02; *P*=0.001; Table 3). Chronic blood transfusions improved functional class status and clinical outcome of clinical right heart failure at 6 and 12 months after treatment. None of the patients had clinical evidence of thromboembolism (deep-vein thrombosis, pulmonary embolism, stroke). No patients were prematurely terminated from the study.

Discussion

In the study reported here, chronic blood transfusions had a significant effect on PASP and mean 6-minute walk distance after 12 months. The results of this study support the treatment of thalassemic patients with PAH with chronic blood transfusions, according to a previous study that showed that correction of hypercoagulability and amelioration of pulmonary arterial hypertension by chronic blood transfusions was seen in an asplenic E/β-Thal patient.[18]

Table 1 Baseline characteristics of the patients[a]

Characteristic	Chronic transfusion, n=16	Occasional transfusion, n=14	*P*-value
Male (n [%])	4 (25.0)	4 (28.6)	1.000
Age (years)	36.1±14.6	34.1±15.2	0.708
Weight (kg)	40.1±8.4	44.2±6.7	0.165
Height (cm)	149.8±7.5	154.1±10.1	0.196
Splenectomy (n [%])	8 (50.0)	4 (28.6)	0.284
NYHA classification (n [%])			
II	11 (68.7)	14 (100.0)	0.045
III	5 (31.3)	0	0.045
Clinical right heart failure* (n [%])	5 (31.3)	3 (21.4)	0.689
Acetyl salicylic acid (n [%])	13 (81.2)	11 (78.6)	1.000
Systolic blood pressure (mmHg)	108.6±10.9	112.9±14.4	0.360
Diastolic blood pressure (mmHg)	64.4±7.8	68.7±11.3	0.235
Pulse rate (/minute)	82.5±12.8	84.2±13.2	0.721
O_2 saturation (%)	95.7±3.2	96.6±4.0	0.306
Hemoglobin (g/dL)	6.6±1.0	6.0±1.3	0.149
Corrected white blood cells ($\times10^3/\mu L$)	10.3±6.4	8.5±4.4	0.454
Platelets ($\times10^3/\mu L$)	413.5±275.5	331.1±328.3	0.270
Nucleated RBC/100 WBC	177.2±225.7	188.1±308.8	0.706
PT (second)	13.8±2.8	13.0±1.2	0.818
PTT (second)	31.8±2.2	30.1±2.8	0.072
INR	1.3±0.2	1.2±0.1	0.250
Aspartate transaminase (IU/L)	66.8±62.3	41.7±24.9	0.096
Alanine transaminase (IU/L)	36.1±27.5	28.1±18.2	0.441
HBs Ag positive (n [%])	1 (6.2)	1 (7.1)	1.000
Anti-HCV positive (n [%])	2 (12.5)	0	0.485
Serum creatinine (mg/dL)	0.8±0.2	0.7±0.2	0.208
Serum ferritin (μg/L)	1,592.8±868.1	1,378.6±649.9	0.456
Echocardiographic finding			
PASP (mmHg)	59.7±15.1	49.0±13.8	0.054
PASP (mmHg) severity (n [%])			
36–50 mmHg (mild)	4 (25.0)	10 (71.4)	0.049
51–70 mmHg (moderate)	8 (50.0)	2 (14.3)	0.058
>70 mmHg (severe)	4 (25.0)	2 (14.3)	0.657
LVEF (%)	62.8±8.5	63.6±8.6	0.807
LVESd (mm)	9.8±2.5	9.9±1.9	0.888
LVEDd (mm)	51.6±4.9	52.0±5.6	0.820
MPAd (mm)	26.7±5.4	25.7±4.4	0.571
RVd (mm)	32.1±10.1	29.9±3.2	0.441
Poor right ventricular systolic function (n [%])	7 (43.7)	2 (14.3)	0.118
Diastolic function			
Mitral valve E:A ratio	1.4±0.5	1.6±0.5	0.337
Mitral valve DT (ms)	200.1±26.7	201.1±6.8	0.884
Baseline 6-minute walk distance (m)	357.5±88.5	371.8±75.5	0.640
Propensity score	0.8±0.3	0.2±0.2	<0.001

Notes: [a]Values shown are mean ± standard deviation unless otherwise specified. *Elevated jugular venous pressure, hepatojugular reflux, and edema.

Abbreviations: DT, deceleration time; E:A, ratio of the early (E) to late (A) ventricular filling velocities; HBs Ag, hepatitis B surface antigen; HCV, hepatitis C virus; INR, international normalized ratio; LVEDd, left ventricular end diastolic diameter; LVEF, left ventricular ejection fraction; LVESd, left ventricular end systolic diameter; MPAd, main pulmonary artery diameter; NYHA, New York Heart Association; PASP, pulmonary artery systolic pressure; PT, prothrombin time; PTT, partial thromboplastin time; RBC, red blood cells; RVd, right ventricular diameter; WBC, white blood cells.

Table 2 Comparison of clinical outcomes, echocardiographic findings, and 6-minute walk distance at 6 and 12 months[a]

Outcome	Chronic transfusion, n=16	Occasional transfusion, n=14	*P*-value
Treatment: red cell transfusion (units/year)	23.9±8.1	4.1±2.2	<0.001
6 months			
Hemoglobin (g/dL)	7.5±0.9	6.2±1.3	0.005
Serum ferritin (μg/L)	1,920.9±795.8	1,635.6±817.7	0.318
Clinical			
NYHA classification (n [%])			
II	16 (100.0)	12 (85.7)	0.209
III	0	2 (14.3)	
Clinical right heart failure (n [%])	1 (6.2)	2 (14.3)	0.586
O_2 saturation (%)	96.0±4.6	96.3±4.0	0.593
Echocardiographic finding			
PASP (mmHg)	50.4±14.6	49.4±16.9	0.308
LVEF (%)	62.2±9.7	61.3±9.0	0.933
MPAd (mm)	27.2±5.2	27.0±5.0	0.842
RVd (mm)	30.2±6.7	30.4±3.9	0.786
Poor right ventricular systolic function (n [%])	2 (12.5)	2 (14.3)	1.000
Diastolic function			
Mitral valve E:A ratio	1.1±0.3	1.4±0.6	0.084
Mitral valve DT (ms)	213.0±16.7	215.3±33.7	0.812
6-minute walk distance (m)	388.7±59.2	368.6±61.1	0.462
12 months			
Hemoglobin (g/dL)	7.2±1.1	5.7±1.6	0.006
Serum ferritin (μg/L)	2,070.7±825.2	1,633.3±1,049.0	0.145
Clinical			
NYHA classification (n [%])			
II	16 (100.0)	13 (92.9)	0.467
III	0	1 (7.14)	
Clinical right heart failure (n [%])	0	1 (7.14)	0.467
O_2 saturation (%)	95.6±5.1	96.3±3.0	0.814
Echocardiographic finding			
PASP (mmHg)	48.8±18.8	54.1±21.4	0.770
LVEF (%)	66.0±7.9	64.8±10.4	0.868
MPAd (mm)	26.7±4.1	27.7±4.8	0.557
RVd (mm)	27.1±7.0	30.0±4.7	0.197
Poor right ventricular systolic function (n [%])	2 (12.5)	2 (14.3)	1.000
Diastolic function			
Mitral valve E:A ratio	1.3±0.6	1.5±0.7	0.288
Mitral valve DT (ms)	195.9±40.1	202.8±11.6	0.369
6-minute walk distance (m)	403.7±53.8	350.0±82.9	0.084

Note: [a]Values shown are mean ± standard deviation unless otherwise specified.

Abbreviations: DT, deceleration time; E:A, ratio of the early (E) to late (A) ventricular filling velocities; LVEDd, left ventricular end diastolic diameter; LVEF, left ventricular ejection fraction; MPAd, main pulmonary artery diameter; NYHA, New York Heart Association; PASP, pulmonary artery systolic pressure; RVd, right ventricular diameter.

Table 3 Effect of chronic blood transfusion on clinical outcomes, echocardiographic findings and 6-minute walk distance

Outcome	Adjusted effect* (95% CI)	*P*-value
6 months		
Clinical		
NYHA class III (n)[a]	−0.37 (−0.37 to −0.37)	<0.001
Clinical right heart failure (n)[a]	−0.37 (−0.37 to −0.37)	<0.001
O_2 saturation (%)[b]	−1.53 (−15.39 to 12.33)	0.829
Echocardiographic finding		
PASP (mmHg)[b]	−6.67 (−16.68 to 3.35)	0.192
LVEF (%)[b]	4.70 (−6.63 to 16.04)	0.416
Poor right ventricular systolic function (n)[c]	0.51 (0.23 to 1.13)	0.099
Diastolic function		
Mitral valve E:A ratio[b]	−0.28 (−1.91 to 1.36)	0.739
Mitral valve DT (ms)[b]	−31.68 (−50.99 to −12.38)	0.001
6-minute walk distance (m)[b]	10.88 (−17.45 to 39.22)	0.451
12 months		
Clinical		
NYHA class III (n)[a]	−0.14 (−0.15 to −0.14)	<0.001
Clinical right heart failure (n)[a]	−0.14 (−0.15 to −0.14)	<0.001
O_2 saturation (%)[b]	3.76 (−10.02 to 17.54)	0.593
Echocardiographic finding		
PASP (mmHg)[b]	−16.83 (−26.35 to −7.32)	0.001
LVEF (%)[b]	3.02 (−8.90 to 14.94)	0.619
Poor right ventricular systolic function (n)[c]	0.51 (0.23 to 1.13)	0.099
Diastolic function		
Mitral valve E:A ratio[b]	0.28 (−1.39 to 1.95)	0.744
Mitral valve DT (ms)[b]	−32.48 (−52.44 to −12.53)	0.001
6-minute walk distance (m)[b]	46.55 (18.08 to 75.02)	0.001

Notes: *Adjusted for propensity score (age, splenectomy, acetyl salicylic acid, NYHA classification, clinical right heart failure, O_2 saturation, baseline pulmonary artery systolic pressure, left ventricular ejection fraction, diastolic function, hemoglobin level, platelet count, nucleated red cell, baseline serum ferritin level, and baseline 6-minute walk test) and right ventricular systolic function. [a]Generalized linear models: extensions to the binomial family: risk difference; [b]generalized linear models: Poisson regression; [c]generalized linear models: extensions to the binomial family: risk ratio.

Abbreviations: CI, confidence interval; DT, deceleration time; E:A, ratio of the early (E) to late (A) ventricular filling velocities; LVEF, left ventricular ejection fraction; MPAd, main pulmonary artery diameter; NYHA, New York Heart Association; PASP, pulmonary artery systolic pressure; RVd, right ventricular diameter.

A previous study showed that PAH is typical in TI patients who do not receive transfusions.[11] PAH in thalassemia has also been associated with platelet activation and a hypercoagulable state.[4] Diverse factors contributing to the hypercoagulable state in patients with thalassemia have been identified.[19] Hypercoagulability is a well-described comorbid state in β-thalassemics who do not receive transfusions.[8] Plasma heparin cofactor II levels were found to be significantly lower in TI patients who did not receive transfusions than in thalassemia major patients who received transfusions.[20]

Management of PAH is symptomatic and supportive to improve right ventricular function. Acetyl salicylic acid[21,22] and an anticoagulant[8,23] are also prescribed by some. Response to treatment is poor and the survival of patients of NYHA functional class III to IV is about 1 to 2 years.

The marked reduction in PASP achieved in our study suggests an improvement in thrombotic pulmonary arteriopathy as a result of chronic blood transfusions.

The preference 14 patients had for occasional transfusions was due to the amount of time required to travel to and from hospital; consequently, they could not manage visiting more than once per month.

The present study may have some limitations as it was not a randomized controlled trial due to ethics considerations, but the propensity score adjustment was applied to cope with such limitations.

Conclusion

The findings of the study presented here suggest that chronic blood transfusions might have beneficial effects on PAH in thalassemia patients over 1 year.

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Disclosure

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