Original Article

Features Associated with Pulmonary Hypertension in Splenectomized Patients with Hemoglobin E/β - Thalassemia Disease

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Abstracts:

Presently, pulmonary hypertension (PHT) has become more convenient to diagnose because of the greater availability of transthoracic echocardiography (TTE). Thalassemia is a major etiology of PHT due to its large population of patients. However, the mechanisms concerning the disease are still unclear and require further investigation. This present study aimed to find factors correlated with PHT in splenectomized thalassemia patients. Sixty-one splenectomized β-thalassemia/Hb E patients were enrolled in the study. Thorough clinical data and laboratory indices were retrospectively reviewed. Among 61 patients, 32 received a diagnosis of PHT by TTE. Pearson's correlation coefficient was used to ascertain the association between factors. Student's t-test or Mann -Whitney test was used for continuous variables, while X^2-test or Fisher exact test was used for categorical ones. Correlation analysis was used to determine the relationship between pulmonary artery systolic pressure (PASP) and selected variables. Blood transfusion requirement along with lower amount of RBCs were significantly associated with high PASP (p-value < 0.05). High levels of serum globulin, lactate dehydrogenase (LDH) and soluble vascular cell adhesion molecules-1 (sVCAM-1) and low level of cholesterol were identified to relate to PHT (p-value < 0.05). In summary, hemolytic anemia and increased sVCAM-1 were noted to link with PHT. To prevent PHT in splenectomized thalassemia patients, adequate blood transfusion to avoid chronic hypoxia might be beneficial.

Keywords : ● *Pulmonary hypertension* ● *Asplenia* ● *Thalassemia*

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บทคัดย่อ

ภาวะความดันโลหิตสูงในหลอดเลือดปอด เป็นภาวะที่พบได้บ่อยมากขึ้นเนื่องจากการเข้าถึงอุปกรณ์ในการวินิจฉัยอย่างเครื่องอัลตราซาวด์หัวใจผ่านทางผนังทรวงอก ได้มากขึ้น โรคที่เกิดจากภาวะเยื่อหุ้มเซลล์เม็ดเลือดแดงผิดปกติ อย่างโรคธาลัสซีเมีย พบว่าเป็นสาเหตุใหญ่ของภาวะดังกล่าว เนื่องจากเป็นโรคที่พบได้บ่อยในประชากร อย่างไรก็ตามข้อมูลที่อธิบายถึงกลไกการเกิดภาวะดังกล่าวนั้น ยังไม่มีอย่างแพร่หลาย งานวิจัยชิ้นนี้จึงมีเป้าหมายเพื่อหาปัจจัยที่ทำให้เกิดภาวะความดันโลหิตสูงในหลอดเลือดปอด ในกลุ่มผู้ป่วยธาลัสซีเมีย เพื่อที่จะหาวิธีป้องกันภาวะดังกล่าวได้ โดยได้รวบรวมผู้ป่วยธาลัสซีเมียที่ตัดม้ามแล้วจำนวน 61 คน มาศึกษาหาปัจจัยต่างๆ ที่เกิดในสัดส่วนที่แตกต่างกันในกลุ่มที่มีภาวะความดันโลหิตสูงในหลอดเลือดปอด และในกลุ่มที่ไม่มีภาวะดังกล่าว พบว่า ภาวะซีดที่เกิดจากเม็ดเลือดแดงแตกตัว และระดับ sVCAM-1 ที่สูง สัมพันธ์กับภาวะความดันโลหิตสูงในหลอดเลือดปอด ดังนั้นความเข้าใจกลไกในการเกิดภาวะดังกล่าวนี้อาจช่วยป้องกันภาวะนี้ได้โดยกำจัดปัจจัยเสี่ยงที่เกิดขึ้นทั้งสอง

คำสำคัญ : ● *Pulmonary hypertension* ● *Asplenia* ● *Thalassemia*

วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต 2560;27:137-43.

Introduction

Pulmonary hypertension (PHT) associated with thalassemia/hemoglobinopathy is now an accepted clinical entity.[1] Depending on the method used, its prevalence varies from a few to over 50% of patients with β-thalassemia disease (β-Thal).[1,2] Currently, the diagnosis is invariably made by transthoracic echocardiogram because it is noninvasive and widely available. As assessed by this method, the entity is the most common cause of PHT worldwide due to a high prevalence of thalassemia/hemoglobinopathy.[3,4] Despite its commonness, its pathogenesis is not yet completely understood. Related studies have shown thrombotic pulmonary arteriopathy, similar to that found in the idiopathic or primary counterpart, as the underlying basis of clinically significant PHT.[5] Formation of this arteriopathy is likely facilitated by the thrombophilic blood profile, activation of vascular endothelial cells as well as decreased nitric oxide bioavailability and dysregulation of arginine metabolism from intravascular hemolysis following splenectomy.[6,7] These aberrations were attributed to an increased amount of red blood cells (RBC) with exposed phosphatidylserine (PS- RBC) and mononuclear phagocyte activation after splenectomy, and help explain the observation that surgical asplenia was a risk factor of PHT in patients with β-Thal.[8-13] Our recent study in 110 adult patients with hemoglobin E/β-thalassemia disease (E/β-Thal), showing a prevalence of PHT in 52.5% and 18.4% (p-value < 0.001) of the splenectomized and the nonsplenectomized patients respectively, was supportive.[14] Because clinically significant PHT was found only in splenectomized patients, knowing the associated features in this group would lead to a better understanding of the pathogenesis, which would ultimately lead to better patient care and improved disease prevention.

Materials and Methods

Splenectomized patients of the previously-reported cohort[14] were selected for study. Altogether 61 patients were enrolled, none of whom had a past or present history, or clinical evidence of venous thrombo-embolic diseases. All were conventionally managed with leukocyte-poor packed red blood cells (LPRC) transfusion, iron chelation therapy and folic acid. Both LPRC transfusion and iron chelation therapy were not aggressive or regular. Iron chelation was performed by a prolonged subcutaneous injection of Desferrioxamine, with only a few receiving Deferiprone in view of its unavailability until late 2006. Blood transfusion and all medications aside from folic acid were not allowed 4 weeks before the study. All patients gave written informed consent, and the study protocol was approved by the institution's ethics committee on studies in humans (# 0774/2548).

Blood for hematology, biochemistry and biomarkers of activation assays, as well as chest roentgenogram and transthoracic echocardiogram were performed as previously described.[14] Pulmonary hypertension was defined as an estimated pulmonary artery systolic pressure (PASP) at rest ≥ 36 mmHg.[15]

Clinical features and laboratory data of the patients, dichotomized according to the presence (PHT+) or absence (PHT-) of PHT, were statistically analyzed. Continuous variables were expressed as mean ± standard deviation or median (ranges), and the categorical ones as proportions. Pearson's correlation coefficient, r, was calculated as part of the preliminary analysis to identify associations among the different variables. Student's t-test or Mann-Whitney test and X^2-test or Fisher's exact test were, respectively, used for continuous and categorical variables to compare characteristics of the two cohorts. Correlation analysis was used to determine the relationship between PASP and selected variables. All analyses were performed by STATA, version 13.0 (Stata Corp, College Station, TX, USA), considering a p-value < 0.05 as statistically significant.

Results

Results expressed in mean ± SD or median (range) of the various measured parameters and their statistically significant differences are shown in Table 1. Four and 57 patients respectively had E/β^+-Thal and E/β^0-Thal

Table 1 Clinical features, chest roentgenogram, and laboratory results of 61 splenectomized hemoglobin E/β - thalassemia disease (E/β - Thal) adult outpatients, dichotomized according to the presence (PHT+) or absence (PHT-) of pulmonary hypertension (estimated PASP at rest ≥ 36 mmHg).

Results	Normal reference values	PHT+ (n = 32)	PHT- (n = 29)	p-value
Diagnosis : E/β^+ - Thal	-	1	3	0.338
E/β^0 - Thal		31	26	
PASP (mmHg)	< 36	48.03 ± 10.98	25.07 ± 6.1	< 0.001
Sex: Female	-	14	17	0.246
Age (years)	-	28.54 ± 10.16	29.83 ± 10.94	0.635
Duration post splenectomy (years)	-	15.08 (0.92-39.42)	14.92 (1.92-40.33)	0.718
Total LPRC transfusion (units)	0	40 (3-452)	21 (5-247)	0.005
Extramedullary hematopoiesis	0	19	10	0.065
RBC (x10^{12}/L)	4.7 ± 0.5	2.98 ± 0.44	3.38 ± 0.62	0.008
Corrected reticulocyte (%)	0.5-2	5.8 (1.0-8.1)	5.2 (0.9-16.9)	0.692
NRBC/100 WBC	0	715.5 (120-2,510)	435 (4-1,330)	0.056
Corrected WBC (x 10^9/L)	6.9 ± 1.6	10.10 ± 3.99	9.28 ± 4.45	0.455
Platelet (x 10^9/L)	178 (119-297)	751.16 ± 235.06	697.96 ± 190.41	0.347
HbF (%)	0	36.86 ± 15.86	34.57 ± 13.94	0.567
Albumin (g/dL)	4.3-5.3	4.26 ± 0.55	4.49 ± 0.36	0.087
Globulin (g/dL)	2.1-2.9	4.78 ± 1.17	4.09 ± 0.73	0.021
Bilirubin (mg/dL): total	0-1	4 (2.2-12)	3.45 (1-7.8)	0.038
direct	0-0.3	0.6 (0.4-1.3)	0.4 (0.2-0.8)	0.008
ALT (U/L)	30-65	75 (16-124)	62 (18-184)	0.489
AST (U/L)	15-37	74 (24-274)	55.5 (9-115)	0.067
Cholesterol (mg/dL)	147-202	88.38 ± 23.36	110.27 ± 24.06	0.003
LDH (U/L)	100-190	345.06 ± 130.80	231.52 ± 106.08	0.006
Serum cell-free Hb (mg/dL)	1.4 (0.6-4.3)	4.68 (2.39-29.4)	3.44 (1.01-13.93)	0.067
Ferritin (μg/L)	10-300	1,950 (424-71,80)	1834.2 (97.9-9,520)	0.696
Anti-HCV	neg	7	2	0.167
hs-CRP (mg/L)	0.5 (0.2-2.8)	3.12 (0.67-41.31)	1,83 (0.32-9.61)	0.068
TAT (μg/L)	2.03 (1.32-8.12)	4.20 (2.07-14.72)	2	0.49
sVCAM-1 (ng/mL)	484.5 ± 99.3	1,920.32 ± 600.60	1.83 (0.32-9.61)	0.001
sE-Selectin (ng/mL)	27.9 ± 9.5	49.26 ± 24.32	3.30 (1.20-11.31)	0.149
sP-Selectin (ng/mL)	130.5 ± 17.9	113.92 ± 29.04	113.08 ± 33.4	0.936
NT pro-BNP (pmol/L)	2.9 (0.6-13.9)	9.21 (2.55-112.80)	5.89 (2.00-21.60)	0.086

*results were expressed as mean ± SD or median (range). ALT, alanine aminotransferase; AST, aspartate aminotransferase; Hb, hemoglobin; HCV, hepatitis C virus; hs-CRP, high-sensitivity C-reactive protein; LDH, lactate dehydrogenase; LPRC, leukocyte-poor packed red blood cells; NRBC, nucleated red blood cell; NT pro- BNP, amino-terminal pro-brain-type natriuretic peptide; PASP, pulmonary artery systolic pressure; sE-Selectin, soluble E-Selectin; sP-Selectin, soluble P-Selectin; sVCAM-1, soluble vascular cell adhesion molecule-1; TAT, thrombin anti-thrombin complexes; WBC, white blood cell.

as shown by identifying Hb A, E, F and E, F in the Hb typing. Thirty-two (52.5%) of 61 patients were PHT+, 14 of whom were female. In addition to sex, age also did not differ (p-value = 0.246 and 0.653, respectively). Duration of postsplenectomy period also did not differ (p -value = 0.718), making any potential effect of this factor on both cohorts balanced. The blood transfusion requirement was higher in the PHT+ cohort (p-value = 0.005). The amount of RBCs was lower in the PHT+ cohort (p = 0.008), while the amount of corrected white blood cells and platelets did not differ (p-value = 0.455 and 0.347, respectively). The amount of platelets in both cohorts; however, was moderately to markedly higher than normal. Hemoglobin F levels did not differ (p-value = 0.567). Serum globulin, lactate dehydrogenase (LDH) and soluble vascular cell adhesion molecule-1 (sVCAM-1) levels were higher (p-value = 0.021, 0.006 and 0.001, respectively), while the cholesterol level was lower (p-value = 0.003) in the PHT+ cohort. None exhibited positive serology for human immunodeficiency virus, and prevalence of anti-hepatitis C virus antibody did not differ (p-value = 0.167). Chest roentgenogram showed no evidence of lung disease in all patients, while extramedullary hematopoiesis was found in 59.4% and 34.5% of the PHT+ and PHT- cohorts, respectively and did not differ (p-value = 0.065). Various degrees of thalassemic bone changes were found in all patients. Transthoracic echocardiogram showed normal left ventricular ejection fraction and no valvular heart disease in all patients, and 32 (52.5%) had an estimated PASP at rest of 48.03 $\pm$ 10.98 or $\geq$ 36 mmHg, i.e., the definition of PHT.

Discussion

The findings suggested no significant role of parenchymatous lung disease, the left heart and extramedullary hematopoiesis in patients' PHT. Hemolysis was more severe in the PHT+ cohort as shown by a higher blood transfusion requirement, lower amount of RBCs, together with higher serum LDH and lower serum cholesterol levels. Only recently was hypocholesterolemia shown to be a feature of chronic anemias with increased erythropoietic activity.[16] An inverse relationship between levels of serum cholesterol and LDH (r = -0.390, p-value = 0.019) in our patients was supportive. The findings suggested a significant role of hemolytic severity in patients' PHT. A higher level of serum sVCAM-1 in the PHT+ cohort (p-value = 0.001) suggested more vascular endothelial cell dysregulation, which could have resulted from thrombin, systemic hyper-inflammatory state, decreased nitric oxide bioavailability from increased serum cell-free Hb, and hypoxemia.[14] Because levels of plasma thrombin antithrombin complexes, serum high-sensitivity C-reactive protein (hs-CRP) and cell-free Hb did not differ (p-value = 0.490, 0.068 and 0.067, respectively), the role of hypoxemia seemed more eminent. Hypoxia has been shown to lead to vascular endothelial activation and altered gene expression in sickle cell disease patients with high stroke risk and PHT, respectively.[17,18] Reduction of the elevated serum sVCAM-1 levels by blood transfusion reaffirms its relationship with hypoxia.[19,20] Taken together, the findings suggested that an increased amount of serum sVCAM-1 from prolonged hypoxia/anemia was essential in the pathogenesis of PHT in patients with splenectomized E/β-Thal despite the pre-existing thrombophilic blood profile.[6] Serum amino-terminal pro-brain-type natriuretic peptide (NT-proBNP) levels did not differ (p-value =0.086). This was unexpected because they have been shown to increase in PHT in proportion to the extent of right ventricular dysfunction.[21] This could be due to a small sample size, concomitant ventricular dysfunction from chronic iron overload as shown by the elevated serum NT-proBNP levels (p-value < 0.001 and 0.001 for the PHT+ and PHT- cohorts, respectively) and the relatively nonsevere PHT. This assumption was supported by the correlation between levels of serum NT-proBNP and PASP (r = 0.34, p-value = 0.040). Levels of PASP, as the dependent variable, correlated significantly with levels of RBCs (r = -0.37, p-value = 0.007),

cholesterol (r = -0.44, p-value = 0.002), LDH (r = 0.52, p-value = 0.001), sVCAM-1 (r = 0.60, p-value = < 0.001), globulin (r = 0.32, p-value = 0.027) and NT-proBNP (r = 0.34, p-value = 0.040). The findings lend additional support to the role of anemia and prolonged hypoxia/anemia as shown by changes in RBC, cholesterol, LDH and sVCAM-1, respectively in the pathogenesis of PHT. A relationship to globulin but not hs-CRP suggested that there could also be a role of chronic low-grade systemic inflammation, consistent with a recent case report.[22] This study is the first to show that status of the spleen affects features associated with PHT. An association between extramedullary hematopoiesis as seen on chest roentgenogram and certain blood changes after splenectomy, such as increased serum cell-free Hb levels, thrombocytosis, and increased amount of nucleated RBC with PHT[14], was lost in this study. Pathogenesis of PHT in patients with splenectomized E/β-Thal is not yet completely understood. An increased amount of PS-RBCs, severe chronic anemia, and mononuclear phagocyte activation have been shown to play a role.[7-10] The role of increased serum sVCAM-1 levels in the present study extends our knowledge and should be verified by further study of a larger cohort.

Summary

In conclusion, features associated with PHT in patients with splenectomized E/β-Thal were more severe hemolytic anemia and increased serum sVCAM-1 levels, likely from more severe chronic hypoxia/anemia. To prevent the disease, a better understanding of the hemolytic mechanism to avoid splenectomy and of the importance of giving adequate blood transfusion to prevent chronic hypoxia, which has a more deleterious effect on these patients, is mandatory. To minimize hazards of blood transfusion, further studies to find the optimal Hb levels are required.

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