Endothelial Function in Beta-Thalassemia Patients

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Abstract

Background: The pathogenesis of pulmonary hypertension in thalassemic patients is unclear and of multifactorial factors. Study in sickle cell disease demonstrated role of endothelial dysfunction in pulmonary hypertension. However, this is not known in Beta-thalassemia patients. This study is to determine endothelial function in Thalassemic patients.

Methods: 26 thalassemia patients aged 18-50 years were recruited and divided into 2 groups: 14 non-splenectomized, and 11 splenectomized patients. There were 7 control cases. Flow mediated dilatation (FMD) of brachial artery was determined as endothelial dependent and endothelial independent (sublingual nitroglycerine). Pulmonary artery systolic pressures was determined non-invasively by Doppler methods. The cardiac function was assessed by transthoracic echocardiography.

Results: There was no significant difference of endothelial function in control group, splenectomized thalassemic patients, and non-splenectomized thalassemic patients using one way ANOVA (FMD 7.96 ± 3.24 %, 8.22 ± 5.45 % and 6.57 ± 3.79%, p 0.628), (post nitroglycerine FMD 24.85 ± 10.21%, 20.64 ± 12.67%, 20.03 ± 10.26%; p 0.650). Pulmonary hypertension was found in both splenectomized (6/11) and non-splenectomized patients (4/14). There was no significant difference of FMD in both pulmonary hypertension subgroups.

Conclusion: Endothelial function does not play major role in thalassemic patients with and without pulmonary hypertension.

Introduction

Pathogenesis of pulmonary hypertension is multifactorial. Endothelial dysfunction could play a role by altering endothelial vasoactive mediators such as NO, prostacyclin, endothelin-1 (ET-1), serotonin, etc. These mediators affect the growth of smooth muscle cells and then facilitate the development of pulmonary vascular hypertrophy, structural remodeling and then pulmonary hypertension (1,2).

Based on the concept of precapillary arterial vasoconstriction that eventually results in morphologic changes of the vessels (remodeling). Testing for pulmonary vasoreactivity using infusion of acetylcholine to determine endothelial function has been studied by previous authors (8).

There is also evidence of an increase in circulating endothelial cells in severe pulmonary hypertension (9). However, whether these cells are shed from vascular lesions or are derived from bone marrow is not clear. Endothelial function had been shown to play an important role in regulation of vascular tone and the propensity of developing cardiac failure (10).

Pulmonary hypertension in thalassemic patients has been viewed to result from various pathogenit mechanisms. In vitro studies have shown disturbances of human vascular endothelial cell function when a cell culture is incubated with thalassemic serum (3,4). One in vivo study demonstrated endothelial dysfunction in patients with β-thalassemia...
major using measurements of pulse-wave velocity in the brachioradial artery (5). This study demonstrated no correlation between serum ferritin and the degree of arterial stiffness. The population in this study had received desferrioxamine which might lead to poor correlation between serum and tissue iron status. The alteration of arterial structures with disruption of elastic tissue (6) and calcification (7) has also been demonstrated in patients with thalassemia supporting the concept of increasing systemic arterial stiffness in thalassemia.

To the best of our knowledge, no studies compared changes in endothelial function in different groups of thalassemic patients. In Thailand, there is a small population of thalassemics that received iron chelating agents. We speculated that the relationships between serum ferritin and cardiac and endothelial dysfunction may differ in this group from previous studies.

Our study aims to determine endothelial function in difference groups of beta-thalassemic patients.

**Methods**

**Study design and population**

We performed a prospective descriptive study of thalassemic patients aged between 15-50 years. They had attended the hematology clinic at Ramathibodi Hospital, Bangkok. Exclusion factors were smoking, heart failure, systemic hypertension, type II diabetes mellitus, thyroid and parathyroid dysfunction, serum creatinine over 2 mg/dl, Hypercholesterolemia or previous recipients of iron chelating agents.

Three investigators participated in this study. They used a SONOS 5500 echocardiographer with vascular probe (11-2L Phillips). We divided subjects in 3 groups; healthy controls, non-splenectomized thalassemics and splenectomized thalassemics.

**Definition of terms**

Thalassemia = abnormal by Hb typing

Pulmonary hypertension = hemodynamically diagnosed by transthoracic echocardiography; systolic pulmonary pressure > 35 mmHg, diastolic pressure >15 mmHg, mean pulmonary artery pressure >25 mmHg(11)

Brachial artery endothelial dysfunction = vasodilatation response to endothelium-dependent and –independent stimuli.

Flow-mediated endothelium-dependent vasodilation = change in brachial artery diameter during reactive hyperemia from baseline.

Flow-mediated endothelium-independent vasodilation = change in brachial artery diameter after 5 minute of 200 mcg sublingual glyceryl trinitrate.

Reactive hyperemia = induced by deflating a cuff previously inflated to 50 mmHg above patient’s systolic BP for 5 minutes.

**Study Protocol**

After the subjects were informed and enrolled to the study protocol, they were instructed to fast and stop caffeine, smoking and physical activity for at least 2 hours before the test. All subjects rested in supine position for at least 10 minutes before blood pressure and cardiovascular measurements were obtained and remained in the supine position throughout data collection. The baseline 2D vascular image of brachial artery diameter was obtained in the right arm before inflating BP cuff to 50 mmHg above systolic pressure for 5 minutes. 2D vascular image was obtained after the cuff deflation as post cuff deflation brachial artery diameter. The parameters were measured 3 times using the average values to calculate flow-mediated dilatation (FMD).

Subjects then rested for 10 minutes post reactive hyperemia and repeat resting 2D image of brachial artery diameter was carried out. They the received 1 oral spray of nitroglycerine 200 mcg to evaluate endothelium independent vasodilation. After a wait of 3 minutes we obtained 2D vascular image post NTG. As reactive hyperemia parameter, we repeated measurements 3 times to obtain average values to calculate FMD.

\[
\text{Flow - mediated Dilatation (FMD) = } \frac{\text{Maximal diameter - Baseline diameter}}{\text{Baseline diameter}} \times 100
\]

Figure 1 shows diagram of the study protocol.

Transthoracic echocardiography was performed to evaluated cardiac function as follows: **M-mode measurements:** Interventricular septum at diastole and systole (IVS d/s), Left ventricular dimension at...
Table 1. Baseline Characteristics

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control N = 7 (mean/SD)</th>
<th>Non-splenectomy N = 15 (mean/SD)</th>
<th>Splenectomy N = 12 (mean/SD)</th>
<th>P value (between group)</th>
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</thead>
<tbody>
<tr>
<td>Age</td>
<td>28.29/6.18</td>
<td>29.13/9.39</td>
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<td>Weight</td>
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<td>52.07/12.87</td>
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<td>Height</td>
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<td>164.57/10.02</td>
<td>159.17/11.42</td>
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<td>BMI</td>
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<td>19.02/2.89</td>
<td>18.12/3.23</td>
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<tr>
<td>HR</td>
<td>71.42/4.75</td>
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<td>SBP</td>
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<td>111.40/13.05</td>
<td>106.58/9.00</td>
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<td>DBP</td>
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<td>65.80/9.70</td>
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<td>Hemoglobin</td>
<td>13.90/0.89</td>
<td>8.57/2.16</td>
<td>7.07/0.79</td>
<td>&lt; 0.01*</td>
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<td>Cholesterol</td>
<td>200/19.47</td>
<td>84.07/19.75</td>
<td>93.83/18.75</td>
<td>&lt; 0.01*</td>
</tr>
</tbody>
</table>

* differences between control group and the other 2 groups

Figure 1. Diagram of study protocol

Ethics and statistical analysis

All patients were informed of potential adverse effects from the study and signed the consent form. This study was approved by the ethics committee of Ramathibodi Hospital.

All parameters were analyzed using SPSS version 11.5 I software. The differences of endothelial function as measure by flow-mediated dilatation (both reactive hyperemia and endothelial independent) were analyzed with one-way ANOVA and p<0.05 indicated statistical significance. Continuous data were represented by mean ± SD.

Results

We recruited 27 thalassemic patients aged 18-50 years: There were 15 non-splenectomized, 12 splenectomized and 7 healthy control.

We found that no significant differences in reactive hyperemia Flow Mediated Dilatation (FMD) and endothelial independent FMD between all three study groups; control group, pre-splenectomy thalassemia patients and post splenectomy thalassemia patient.
Table 2. Measurement parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control N = 7 (mean/SD)</th>
<th>Non-splenectomy N = 15 (mean/SD)</th>
<th>Splenectomy N = 12 (mean/SD)</th>
<th>P value (between group)</th>
</tr>
</thead>
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<tr>
<td>IVSd</td>
<td>0.67/0.09</td>
<td>0.91/0.13</td>
<td>0.82/0.02</td>
<td>P= 0.008*</td>
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<tr>
<td>IVSs</td>
<td>0.91/0.20</td>
<td>1.27/0.28</td>
<td>1.14/0.25</td>
<td>P= 0.017*</td>
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<tr>
<td>LVIDd</td>
<td>3.92/0.77</td>
<td>5.02/0.54</td>
<td>5.10/0.51</td>
<td>P&lt; 0.01*</td>
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<tr>
<td>LVIDs</td>
<td>2.63/0.47</td>
<td>3.29/0.54</td>
<td>3.39/0.48</td>
<td>P&lt; 0.01*</td>
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<td>LVPWd</td>
<td>0.83/0.18</td>
<td>0.98/0.14</td>
<td>0.90/0.15</td>
<td>P= 0.108</td>
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<td>LVPWs</td>
<td>1.23/0.23</td>
<td>1.42/0.21</td>
<td>1.35/0.21</td>
<td>P= 0.178</td>
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<td>LVEF</td>
<td>61.43/8.00</td>
<td>66.63/8.27</td>
<td>65.18/8.60</td>
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<td>E/A ratio</td>
<td>1.62/0.32</td>
<td>1.54/0.36</td>
<td>1.84/0.66</td>
<td>P= 0.264</td>
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<tr>
<td>PASP</td>
<td>15.28/6.7</td>
<td>29.4/6.85</td>
<td>34.00/10.15</td>
<td>P&lt; 0.01*</td>
</tr>
<tr>
<td>FMD cuff</td>
<td>7.96/3.24</td>
<td>8.23/5.45</td>
<td>6.57/3.79</td>
<td>P= 0.628</td>
</tr>
<tr>
<td>FMD NTG</td>
<td>24.85/10.22</td>
<td>20.64/12.68</td>
<td>20.04/10.26</td>
<td>P= 0.650</td>
</tr>
</tbody>
</table>

* differences between control group and the other 2 groups

We also analyzed differences of FMD between the patient with and without pulmonary hypertension (Table 2), we found that there are no differences of FMD in both groups (Table 3, Figure 2, Figure 3).

Furthermore, from our study there are pulmonary hypertension in both splenectomized and non-splenectomized thalassemic patients.

**Discussion**

Previous studies demonstrated that increased arterial stiffness, endothelial dysfunction, and LV hypertrophy occur in patients with beta-thalassemia major. This may result in reduction of mechanical efficiency of the heart (5). However, these study did not showed whether endothelial dysfunction played a role in causing pulmonary hypertension as has been seen in patients with sickle cell anemia (13). Endothelial dysfunction may contribute in to arterial stiffness, given the important role of endothelium-derived nitric oxide as an inhibitor of smooth muscle contractility. Functional disturbances of human vascular endothelial cells, when incubated with thalassemic serum, have been demonstrated in vitro.

These include an increase in levels of soluble adhesion molecules in the supernatant of cell culture.(14) Recently, soluble adhesion molecules including intercellular adhesion molecule 1, vascular adhesion molecule 1, and E-selectin have been shown to be significantly elevated in the plasma of thalassemia patients (15). The present study provides evidence.

**Figure 2.** Correlation between PA systolic pressure and reactive hyperemia FMD
that arterial endothelial dysfunction occurs in vivo in patients with beta-thalassemia major.

Our study showed no differences in endothelial function between thalassemia patients with and without pulmonary hypertension, with and without splenectomy. We concluded that endothelial dysfunction did not play a major role in thalassemia patients with pulmonary hypertension, and may be a consequence of the disease process. Additionally, we found that pulmonary hypertension also occurs in thalassemia patients prior to splenectomy and that splenectomy did not affect endothelial function and occurrence of pulmonary hypertension in thalassemic patients. This could indicate that the properties of red blood cells after splenectomy may not be a major factor in the etiology of pulmonary hypertension in these patients. The only different

### Table 3. Flow-mediated dilatation according to Pulmonary Artery Systolic Pressure

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Patient without pulmonary hypertension (n = 18)</th>
<th>Patient without pulmonary hypertension (n = 9)</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMD cuff</td>
<td>8.18 ± 5.42</td>
<td>6.11 ± 2.91</td>
<td>7.96 ± 3.25</td>
<td>P = 0.521</td>
</tr>
<tr>
<td>FMD NTG</td>
<td>19.64 ± 10.42</td>
<td>21.84 ± 13.86</td>
<td>24.85 ± 10.22</td>
<td>P = 0.587</td>
</tr>
</tbody>
</table>

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**Figure 3.** Correlation between PA systolic pressure and FMD post NTG

**Figure 4.** Correlation between Hemoglobin levels and PASP

**Figure 5.** Correlation between Cholesterol levels and PASP
factors between study groups were hemoglobin and cholesterol levels. These did not correlate with pulmonary artery pressures (Figure 4, Figure 5).

However, our study has some limitation about factors affecting measurement of FMD. There are differences of hemoglobin levels and cholesterol level in control group and thalassemic patient that can’t be modified due to the natural history of thalassemic disease that may affect the value of Flow-Mediated dilatation.

In conclusion, we demonstrated that in vivo endothelial function and splenectomy did not play a role in the pathogenesis of pulmonary hypertension in thalassemia patients.

Limitations of this study: There have been no previous data on the association between endothelial function of the brachial artery and pulmonary artery.

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Ass. Prof. Piyamitr Sritara

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جانอนิส วิโรจน์, วราวุธ อโศก, วัชรินทร์ บุญมา, ศรีสุรินทร์ ตัณทัชนีย์, วราวุธ อโศก, วัชรินทร์ บุญมา

นี่ยำผลวิจัย การศึกษาการทำงานของ endothelial cell ในผู้ป่วยβ-thalassemia minor พบว่า ที่มีความดันในปอดสูง (pulmonary hypertension) ในโรงพยาบาลรามขันติ

บทคัดย่อ

วัตถุประสงค์: การระคายเคืองในปอดสูงเป็นการกระทบซ่อนที่สำคัญในผู้ป่วยβ-thalassemia minor ซึ่งหากคุณที่ทำให้เกิดการตกค้างชัวร์ไม่เป็นที่ทราบแน่ชัวร์จากการศึกษาที่มีอยู่ในปัจจุบันและซึ่งจะทำให้มีปัจจัยที่สำคัญของกลุ่มปัจจัย การศึกษาที่มีอยู่เป็นการศึกษาในผู้ป่วย sickle cell disease ได้แสดงให้เห็นถึงการทำงานของ endothelial cell ที่สำคัญในการเพิ่มให้เกิดความดันในปอดสูง เมื่อมีกลุ่มปัจจัยที่เกี่ยวกับการทำงานของกลุ่มติดต่อกัน การศึกษามีผลจัดกลุ่มที่สำคัญว่าการทำงานของ endothelial cell มีผลต่อการกักกันความดันในปอดสูงในผู้ป่วยβ-thalassemia minorอย่างไร

วิธีการศึกษา: มีผู้ป่วยเข้าร่วมการศึกษา 26 ราย อายุระหว่าง 18-50 ปี จากกลุ่มโรคติดต่อโรคพยาบาลสามารถหาได้ผู้ป่วยเป็น 2 กลุ่มได้แก่กลุ่มที่ได้รับการดัดแปลง 14 ราย และไม่ได้รับการดัดแปลง 11 ราย ขนาดกลุ่มที่เข้าร่วมการศึกษาเป็นกลุ่มควบคุม 7 ราย ทำการวัดระดับความดันของกลุ่มติดต่อกัน (brachial artery) ต่อสังจากกลุ่ม Flow mediated dilatation (FMD) โดยแยกเป็น endothelial dependent FMD (วัดจาก reactive hyperemia โดยใช้ cuff pressure) และ endothelial independent FMD (วัดจากการพ่น sublingual nitroglycerine) ทำการตรวจวัดคึ้มสูงระดับของความดัน echocardiography เพื่อวัดความดันในปอด (Pulmonary artery systolic pressures) โดยใช้ doppler และการทำงานของหัวใจอย่างอื่นทาง transthoracic echocardiography

ผลการศึกษา: จากการศึกษาพบว่าไม่มีความแตกต่างของการทำงานของ endothelial cell ระหว่างผู้ป่วยกลุ่มควบคุมและผู้ป่วยβ-thalassemia minor โดยใช้วิธีทางสถิติของ one way ANOVA (FMD 7.96±3.24 %, 8.22±4.54 % and 6.57±3.79 %; p 0.628), (post nitroglycerine FMD 24.85±10.21 %, 20.64±12.67%, 20.03±10.26 %; p 0.650) และไม่มีความแตกต่างของ FMD ระหว่างผู้ป่วยβ-thalassemia minorและโรคติดต่อกัน แต่สำหรับคำว่า ศึกษาพบว่าความดันในปอดสูงได้ต่างจากกลุ่มติดต่อกันไม่มีผลติดต่อกัน

สรุป: การทำงานของ endothelial cell ไม่ใช่ปัจจัยหลักที่จะทำให้เกิดความดันในปอดสูงในผู้ป่วยβ-thalassemia minor

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