ORIGINAL ARTICLES

Thrombocytosis in Splenectomized β- Thalassemia Children and its Association With Pulmonary hypertension

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ABSTRACT

**Background:** Pulmonary hypertension (PH) was frequently observed in children with β-thalassemia. Some studies have suggested higher incidence of PH in splenectomized thalassemia children. However, the exact reason for this higher incidence was not clear. **Aim:** To study the occurrence of PH in both splenectomized and non-splenectomized β-thalassemia children and its association with high platelet count (>600 ×10^9 per liter).

**Methods:** 82 patients with β-thalassaemia major were recruited in this study. Pulmonary artery pressure was estimated using echocardiography that was performed by echocardiographer blinded to patients' clinical and laboratory data. Pulmonary artery pressure was estimated from tricuspid regurgitation, pulmonary regurgitation or time to peak velocity of pulmonary flow Doppler flow tracing. Patients with and without PH were compared as regard history of splenectomy and platelet count.

**Results.** 65 patients had PH and 17did not. Patients with PH had significantly more nucleated red blood cells and higher platelet counts. Splenectomy was more prevalent in children affected by PH compared to children without PH (75.8% vs. 25.6%). In multivariate analysis, splenectomy was the only factor significantly related to high platelet count and to PH. **Conclusion:** Higher platelet count was associated with PH in splenectomized children with β-thalassemia.

**Key words:** β-thalassaemia major, Pulmonary hypertension, Splenectomy, Thrombocytosis.

Background:

Heart disease is a major cause of mortality and morbidity in patients with β-thalassemia after the first decade of life despite improved prognosis with iron chelation. The common cardiac abnormalities that have been reported in patients with β-thalassemia are cardiac hypertrophy, ventricular systolic dysfunction, pericarditis and pulmonary hypertension (PH) (Aessopos et al., 2001). PH is found in about 59–75% of patients with β-thalassemia and can be the leading cause of heart failure in these patients. Factors affecting pulmonary artery pressure (PAP) in β-thalassemia patients include; high cardiac output caused by anemia, left ventricular (LV) systolic dysfunction, chronic pulmonary hemosiderosis, recurrent respiratory tract infections, hypoxemia and pulmonary fibrosis. Another proposed cause is the hypercoagulable state with thrombotic obstruction of the pulmonary arteries (Du ZD et al., 1997).

Although most of the reported β-thalassemia patients with PH were splenectomized, non-splenectomized patients can also have PH whereas some of them have normal PAP (22). The relationship between splenectomy and PH in β-thalassemia is now being clearly established. A proposed mechanism involves abnormal red cell membrane phosphatidylserine exposure, which can trigger low-grade hypercoagulability, which is enhanced in the absence of spleen (Cappellini et al., 2000). Alternatively, growing evidence suggests hemolysis-induced nitric oxide scavenging, causing platelet activation, thrombosis, and endothelial dysfunction (9). Although, splenectomy was proposed as a risk factor for chronic thromboembolic PH even in healthy splenectomized individuals, suggesting a general increased risk, this mechanism is possibly enhanced in chronic hemolytic anemia (Ruf et al., 1997).

Thrombocytosis is a known postsplenectomy consequence and is a leading cause for thrombosis and embolic manifestations (Tavazzi et al., 2001). Other biologic risk factors for thrombosis than splenectomy in β-thalassemia children include red cell phosphatidylserine exposure, and plasma coagulation factor abnormalities (Grady et al., 1994). However, the effect of long-standing absence of spleen and thrombocytosis on the development of PH in thalassemia has not been explored.

The purpose of this study is to explore the relationship between PH and thrombocytosisin splenectomized thalassemia diseased children. Data of this work was accepted as a poster presentation in the 26th International Pediatric Association (IPA) congress that held on 4-9 August 2010 in Johannesburg, South Africa.
Methods:

Study Design:

Study population:

In a cross-section study design we prospectively enrolled 82 children aged between 16 months to 16 years diagnosed with thalassemia in the pediatric hematology clinic at Sohag University Hospital, Sohag, Egypt. The study was done in the period from December 2008 to the end of May 2009. 66 patients were males and 16 were females. 74 patients had β-thalassemia major (TM) and received regular transfusions; 8 patients had β-thalassemia intermedia (TI) and received only occasional or no transfusions. Age, spleen status, transfusion state, and history of a thrombotic event were recorded. We included thalassemia children who were free from cardiac symptoms and had no evidences of clinical heart failure or signs of chronic liver disease. None were taking cardio-active drugs at the time of examination. We excluded patients with significant valvular heart disease, congenital heart disease, more than mildly diminished LV systolic function (LVEF) less than 40%, documented cardiac arrhythmia, known risk factors for secondary PH (chronic pulmonary diseases, asthma, and recurrent pulmonary infections) and poor echocardiographic imaging. The study protocol is approved by the institutional research ethics committee. All patients or their legal guardians signed a written informed consent.

Study Procedures:

The 82 participants were examined by echocardiography. Only one investigator, who was kept blinded to the patient’s clinical information, did echocardiographic examinations. Splenectomy status was hidden before and during echocardiographic examinations by covering the upper abdomen in all participants. Clinical data including additional history and complete physical examination were recorded after the echocardiographic examination was completed.

Echocardiographic examination:

Cardiac ultrasound and Doppler examination was done using Acuson (Mountain View, CA) 128/XP10 ultrasound scanner equipped with 5–7 MHz vector array pediatric transducer incorporating color flow and pulsed Doppler. Structural normality of the heart was established on the initial scan. For the purpose of the study, each examination was performed in the following order: Doppler examination of RV inflow across tricuspid valve, Doppler examination of RV outflow across pulmonary valve, and finally M mode examination of right and left ventricle dimensions. RV inflow was examined from standard apical four-chambers view. Flow across the tricuspid valve was determined by color Doppler and then pulsed Doppler sample volume was placed at the tip of the tricuspid valve in diastole to obtain the tricuspid Doppler flow velocity time signal. RV outflow was examined from standard parasternal short axis view. Pulmonary flow was determined by color Doppler and then the pulsed Doppler sample volume was placed in the center of the main pulmonary artery immediately distal to the pulmonary valve to obtain the pulmonary artery Doppler flow velocity time signal. No angle correction was needed for Doppler examination since the angle of intonation was near to zero for both tricuspid and pulmonary flows. The waveform was considered optimal when the highest velocity with a clear distinct envelope was obtained for a minimum of 10–20 consecutive cardiac cycles. M-mode examination was done from the standard parasternal long axis view according to the guidelines of the American Society of Echocardiography for M-mode echocardiography (Sahn et al., 1978). The M-mode beam was placed perpendicular to the interventricular septum at a level transecting the tips of the mitral leaflets, and measurements were taken by the leading edge methodology.

Main endpoints, data acquisition and processing:

For the purpose of volumetric assessment of RV inflow and outflow, velocity time integral of tricuspid (VTItv; cm) and pulmonary (VTIpa; cm) Doppler flow velocity wave form were calculated respectively. Since stroke volume (blood flowing in a single heart beat) is equal to the product of VTI and the cross-sectional area of the orifice it crosses, changes in VTI will directly reflect changes in stroke volume, as long as the changes in cross-sectional area of the orifice of interest is not expected to change (13, 15). Tricuspid and pulmonary valves in study subjects were healthy and it was unlikely that their cross-sectional areas would change between the two examinations. Consequently, VTItv and VTIpa could be used as relative measures of RV inflow and outflow, respectively. RV diastolic function was assessed using RV early to atrial (E/A) filling velocity ratio of tricuspid
valve flow. The lower the E/A ratio the more the impairment of the RV diastolic function (Yu CM et al., 1996). To assess for RV dilatation, RV end-diastolic diameter (RVEDd) from M-mode examination, was measured. Left ventricular global systolic function was assessed using left ventricle fraction shortening (FS) from M-mode examination. Heart rate was also recorded. From the pulmonary artery Doppler flow tracing time to peak velocity of pulmonary flow (TPV) was measured and mean pulmonary artery pressure (PAPm) was calculated using the formula: PAPm [mmHg] = 73 - (0.42TPV) (7). For each parameter, the average of five consecutive cardiac cycles was taken.

Statistical analysis:

Continuous variables were expressed as mean (SD). Student’s t test was used to compare variables between groups. Correlation co-efficient and linear regression were used to identify potential relationship between platelet count and PAPm in splenectomized thalassemia diseased children. Data were statistically analyzed with the SPSS V.10.0 (SPSS Inc, Chicago, Illinois, USA) statistical software package. Statistical significance was taken as a value of two-tailed p < 0.05.

Results:

Of the 82-thalassemia patients, 66 were males and 16 were females their ages range from 16 months to 16 years. 74 patients had TM and 8 had TI. The hemoglobin concentration varied from 9.86 to 3.2 g/l (mean 7.12). History of blood transfusions in the previous 12 months was recorded for all of the 82 patients. The average number of times of blood transfusion was 7.4 times (range 1-13) in one year. Iron chelation was not given to any of our examined patients. Mean LVEF was 68.5% (range 57-78%).

Of the 82-thalassemia patients, 65 child (79.3%) had significant PH (PAPm was more than 30 mmHg). None of the patients had clinical evidence of thromboembolism such as deep vein thrombosis or pulmonary embolism. Thirty-four child had splenectomy operation and 48 child had no splenectomy operation. A comparison of the clinical variables and echocardiographic findings between splenectomized and nonsplenectomized thalassemia patients was presented in Table (1).

In none-splenectomized group PH was significantly associated with age of the child (p<0.01), average pre-transfusion hemoglobin level over last 12 months (p<0.05), and diastolic LV function (measured as mitral valve E/A ration; p<0.05)), while in splenectomized group, PH was significantly associated with units of blood transfusion during the preceding 12 months (p<0.01), time since splenectomy (p<0.05), platelet count (p<0.001), and nucleated RBCs count (p<0.001).

Table 1: A comparison between the clinical variables and echocardiographic findings between thalassemia patients with and without splenectomy.

<table>
<thead>
<tr>
<th>Clinical variables</th>
<th>splenectomy (n=34)</th>
<th>non-splenectomy (48)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mos)</td>
<td>157.5(45.9)</td>
<td>90(61.3)</td>
<td>0.10</td>
</tr>
<tr>
<td>Male</td>
<td>22</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>12</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>TM</td>
<td>34</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>TI</td>
<td>0</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Hb (g/L)</td>
<td>6.7(1.49)</td>
<td>7.4(1.7)</td>
<td>0.06</td>
</tr>
<tr>
<td>Platelet count(x10^9 cells/L)</td>
<td>823.2(477.5)</td>
<td>404.5(168.8)</td>
<td>0.00</td>
</tr>
<tr>
<td>Number of blood transfusion in last year</td>
<td>8.9</td>
<td>2.6</td>
<td>6.25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Echocardiographic findings</th>
<th>splenectomy (n=34)</th>
<th>non-splenectomy (48)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TR</td>
<td>29.3(12.57)</td>
<td>20.4(9.96)</td>
<td>0.00</td>
</tr>
<tr>
<td>LVEF(%)</td>
<td>68.7(5.4)</td>
<td>68.4(6.46)</td>
<td>0.83</td>
</tr>
<tr>
<td>LVEDd (mm)</td>
<td>5.37(8.3)</td>
<td>3.67(0.726)</td>
<td>0.11</td>
</tr>
<tr>
<td>Mitral valve E:A ratio</td>
<td>2.17(0.94)</td>
<td>1.98(0.57)</td>
<td>0.27</td>
</tr>
<tr>
<td>TPV (ms)</td>
<td>73.3(23.86)</td>
<td>96.4(14.8)</td>
<td>0.00</td>
</tr>
<tr>
<td>PAPm (mmHg)</td>
<td>42.2(23.86-51.58)</td>
<td>33.1(19.66-46.96)</td>
<td>0.00</td>
</tr>
<tr>
<td>RVEDD</td>
<td>1.68(0.39)</td>
<td>1.465(0.32)</td>
<td>0.01</td>
</tr>
<tr>
<td>E/A (Right sided)</td>
<td>1.185(0.35)</td>
<td>1.68(0.5)</td>
<td>0.00</td>
</tr>
</tbody>
</table>

TM: Thalassemia Major, TI: Thalassemia Intermedia, Hb: Hemoglobin, TR; LVEF: left ventricle , LVEDd: Left ventricular end-diastolic diameter, mitral E/A: early to atrial filling velocity ratio of mitral valve flow, TPV: time to peak velocity of pulmonary flow, PAPm: Mean Pulmonary Artery Pressure.

Of 34 splenectomized thalassemia patients, 27 had significant PH. A comparison of clinical variables between patients with PH and those without PH in splenectomized thalassemia children is presented in Table (2). In splenectomized thalassemia children platelet count was significantly higher (p-value = 0.01) in patients with PH than in those without PH.
Table 2: A comparison of clinical variables between patients with pulmonary hypertension and those without pulmonary hypertension in splenectomized thalassemia children.

<table>
<thead>
<tr>
<th>Platelet count (x10⁹ cells/L)</th>
<th>*PH (PAPm&gt;30mmHg) n=27</th>
<th>*Non PH (PAPm&lt;30mmHg) n=7</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (months)</td>
<td>109.4 (48.5)</td>
<td>123.4 (35.1)</td>
<td>0.5</td>
</tr>
<tr>
<td>Age at diagnosis (months)</td>
<td>8.5 (7.9)</td>
<td>31.9 (13.4)</td>
<td>1.1</td>
</tr>
<tr>
<td>Male</td>
<td>19</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>8</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Age at splenectomy (months)</td>
<td>68.2 (28.5)</td>
<td>66 (14.3)</td>
<td>0.8</td>
</tr>
</tbody>
</table>

*Values are expressed as mean (SD)

The correlation between platelet count and mean pulmonary artery pressure (PAPm) was strongly positive ($r=0.67$) in splenectomized thalassemia patients and weak positive ($r=0.18$) in non-splenectomized ones. These data were presented as linear regression in Figure (1), and (2).

**Fig. 1:** Scattered graph of the platelet count ($x10^9$) in splenectomized thalassemia children versus mean pulmonary artery pressure (PAPm in mm Hg) for each patient with fitted values estimated by linear regression.

**Fig. 2:** Scattered graph of the platelet count ($x10^9$) in non-splenectomized thalassemia children versus mean pulmonary artery pressure (PAPm in mm Hg) for each patient with fitted values estimated by linear regression.

**Discussion:**
In the present study we hypothesis that thrombocytosis has a role in development of PH in splenectomized thalassemia children. Many studies have shown that PH is a complication of splenectomy in β-thalassemia children (Phrommintikul et al., 2006; Aessopos et al., 2001; Du ZD et al., 1997). The possible underline mechanisms are many and some of them are still need to be proven (Atichartakarn et al., 2003; Carnelli et al., 2003; Eldor and Rachmilewitz, 2002; Aessopos et al., 2001, Sonakul and Fucharoen 1992). High cardiac output caused by anemia (Aessopos et al., 2001), LV systolic dysfunction, chronic pulmonary hemosidrosis, recurrent respiratory tract infections, hypoxemia and pulmonary fibrosis can be possible mechanisms (Zakynthinos et al., 2001; Factor et al., 1994).

On the other hand, thromboembolism as a part of the hypercoagulable state in thalassemia can be another possible one (Cappellini et al., 2000, Sonakul and Fucharoen 1992). Elevation of p-selectin, and decrease serum level of protein C are demonstrated in some studies to be responsible for the hypercoagulable state in thalassemia patients and consequently increasing the risk of pulmonary thrombosis or embolism leading to PH (Eldor and Rachmilewitz 2002; Ciceri et al., 2006). Other studies suggested that elevated p-selectin could be a marker for PH in thalassemia patients and still under-trial if p-selectin inhibitors can decrease elevated PA pressure in thalassemia patients (Factor et al., 1994, Caron et al., 2002; Myers et al., 2005).

Thrombocytosis is a known postsplenectomy consequence (Hoepet et al., 1999; Rostagno et al., 1991); however, the effect of long-standing absence of spleen and thrombocytosis on increased platelet activation and development of PH in thalassemia has not been explored.

In our analysis we determined that in none-splenectomized thalassemia group PH was significantly associated with age of the child (p<0.01), average pre-transfusion hemoglobin level over last 12 months (p<0.05), and diastolic LV function (measured as mitral valve E/A ration; p<0.05), while in splenectomized group, PH was significantly associated with units of blood transfusion during the preceding 12 months (p<0.01), time since splenectomy (p<0.05), platelet count (p<0.001), and nucleated RBCs count (p<0.001) Table (1). We also found that in splenectomized thalassemia children platelet count is significantly higher in PH patients than in patients with normal PAP (Table 2). This finding can confirm that thrombocytosis may be a leading cause of PH in splenectomized thalassemia children. We proved also that there is a strong positive correlation between platelet count and PAPm in splenectomized thalassemia children and this correlation is weak positive in non-splenectomized thalassemia children. These results agree with the results of Singer et al., (2006) which proved that platelet activation and thrombocytosis is associated with PH in splenectomized thalassemia children.

Conclusion:

Our study shows that thrombocytosis is associated with PH in splenectomized β-thalassemia children and further studies should be directed to the underline mechanisms of platelet activation and thrombocytosis to allow detection of serum markers that can help in diagnosis of PH in thalassemia children. Future trials can help in optimizing the treatment of PH, through inhibition of platelet activation and minimizing the hypercoagulability state.

References