Subclinical haemorrhagic tendency exists in patients with β-thalassaemia major in early childhood
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Abstract

Background
Alterations of coagulation profile have been reported in patients with β-thalassaemia major (β-TM).

Method
To investigate this in the paediatric population, we studied haemostatic parameters in pre-transfusion blood samples from 50 non-splenectomised transfusion-dependent children with β-TM (mean age 6±2.5 years) and in blood from 25 healthy controls.

Results
Laboratory evaluation showed thrombocytopenia in 40%, prolongation of prothrombin time (PT) in 12% and prolongation of activated partial thromboplastin time (APTT) in 6% of the patients. Mean values for PT, APTT and platelet count (PC) were all raised in the patient population compared with the controls. The alteration of coagulation status was significant for PT (p value <0.005) and APTT (p value <0.0001). However, the change for PC was not significant (p value >0.05). No significant liner correlation could be identified between PT, APTT, PC of the patients and interval between transfusions (in days) or days since last transfusion.

Conclusion
The findings from this study suggest that a subclinical haemorrhagic tendency exists in patients with β-TM at a very early age. The intrinsic pathway appears to be more affected than the extrinsic pathway.

Key Words
Thalassaemia Major, Haemorrhage, Coagulation, Prothrombin Time, Activated Partial Thromboplastin Time.

What this study adds:
1. Paediatric patients with β-thalassaemia major have a subclinical haemorrhagic tendency.
2. The intrinsic contact activation pathway is more affected in these patients than is the extrinsic tissue factor pathway.
3. Chelation therapy in these patients may further alter the coagulation profile.

Background
Thalassaemia syndromes represent one of the most common forms of hereditary haemolytic anaemia. Presently, over 250 million people worldwide are affected by thalassaemia and allied disorders. The frequency of β-thalassaemia in India ranges from 3.5 to 15% in the general population, with nearly 10,000 children with β-thalassaemia major (β-TM) born every year.1,2 Comprehensive thalassaemia care services have substantially improved life expectancy and quality of life in these patients. Consequently, late effects of the treatment modalities, such as polytransfusion, splenectomy and chelation therapy are gradually becoming more apparent.

The adverse effects on the cardiovascular, endocrine and hepatic systems have been well studied. An emerging body of evidence has drawn attention to haemostatic alterations and their consequent effects. A higher incidence of thromboembolic manifestations has been noted in patients with β-TM and the presence of haemostatic abnormalities suggest the existence of a chronic hypercoagulable state.3–5 These manifestations range from transient ischaemic attack, stroke, acute myocardial infarction, deep vein thrombosis to pulmonary embolism.3,6–8 Inadequate transfusions, a common problem for thalassaemic patients in developing countries, and splenectomy, a standard management modality, have both been implicated in the development of this hypercoagulable state.9 There have also been reports of epistaxis and haematuria, suggesting a haemorrhagic tendency in these patients.10–12 However, most of these studies were performed on adult or young adult
populations. Hence, this study was designed to investigate the situation in a cohort of paediatric patients.

**Method**

This was a cross-sectional laboratory study involving 50 paediatric patients with β-TM and 25 suitable controls (healthy children attending a vaccination clinic, mean age 3.3±1.9 years). Transfusion-dependent (requiring transfusions at intervals of at least 15 days), non-splenectomised children below 10 years of age who had never undergone chelation therapy were included in the study. Children with hepatitis B, C or HIV were excluded. The rationale behind such an inclusion criteria was that coagulation abnormalities have been found in adult patients and children who have had splenectomy and who were receiving chelation therapy. Hence, we wanted to investigate this problem in a cohort of treatment-naive paediatric patients, who were as close to the natural history of the disease as possible. We chose a margin of at least 15 days since the last transfusion in order to eliminate any confounding effects from a recent blood transfusion.

We used the cardinal parameters of haemostasis—prothrombin time (PT, normal 11–16 s), activated partial thromboplastin time (APTT, normal 26–40 s) and PC (platelet count normal 150–400×10³ per µL) to investigate the coagulation profile. Blood was collected from patients (pre-transfusion samples) and controls in sodium citrate (3.2%) and EDTA vials (1.5 mg/mL of blood). An automated blood cell counter (Sysmex Hematology Analyzer KX-21) and coagulation analyser (Sysmex CA-50) were used to determine the PC, PT and APTT. Simple statistics (mean and standard deviation [SD]), Pearson’s coefficient of correlation and multiple hypothesis testing (at level α=0.05) were used in MatLab 7.1 to analyse the data. Informed consent was collected from the guardians of the patients in accordance with the Declaration of Helsinki. The study was approved by the Indian Council of Medical Research (Short Term Studentship program) and the Institutional Ethics Committee of the hospital.

**Results**

The mean age of the patients was 6±2.5 years (range 1.5–10 years). The majority (40%) of the patients required transfusions at intervals of 30 days (Figure 1).

![Figure 1: Proportion of patients grouped according to interval between transfusions](image)

There were significant differences in the PT and APTT values between the patients and controls (Table 1).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients</th>
<th>Controls</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT (s)</td>
<td>14±7</td>
<td>11±1</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>APTT (s)</td>
<td>31±5</td>
<td>26±2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PC (×10³/µL)</td>
<td>217±160</td>
<td>171±22</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

**Table 1: Coagulation parameters in cases and controls (approximate whole number values have been presented)**

The percentage of patients and controls with coagulation parameters outside the normal range are shown in Table 2. Note the percentage of patients with prolonged PT, APTT and thrombocytopenia. A substantial portion of the controls had PT, APTT values below normal range.

<table>
<thead>
<tr>
<th>Coagulation parameter values</th>
<th>Percentage of patients</th>
<th>Percentage of controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Above normal</td>
<td>Below normal</td>
</tr>
<tr>
<td>PT</td>
<td>12%</td>
<td>6%</td>
</tr>
<tr>
<td>APTT</td>
<td>6%</td>
<td>0%</td>
</tr>
<tr>
<td>PC</td>
<td>6%</td>
<td>40%</td>
</tr>
</tbody>
</table>

**Table 2: Percentage of patients and controls with coagulation parameters outside the normal range**

The lack of linear correlation between the coagulation parameters and interval between transfusions and days since transfusion for the patients is shown in Table 3.
in p value for APTT (p<0.0001) than that for change in PT (p<0.005). APTT is the performance indicator for the intrinsic (or contact activation) pathway and the final common pathways whereas PT measures the same for the extrinsic (or tissue factor) pathways and final common pathways. Hence, a more significant change for APPT apparently suggests that the intrinsic pathway may be more consistently affected than the extrinsic pathways. Other authors have also supported this hypothesis of chronic activation of the intrinsic coagulation cascade in these patients owing to the effect of multiple transfusions and haemolysates.\textsuperscript{13,14}

Table 4 shows some pertinent differences between our findings and those by Naithani et al,\textsuperscript{12} who had a very comparable patient population. However, all of their patients were undergoing chelation therapy. None of our patients had ever received chelation therapy; hence, these differences suggested that chelation therapy might confer increased risk for haemostatic alterations.

<table>
<thead>
<tr>
<th>Findings by Naithani et al</th>
<th>This study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombocytopenia seen in</td>
<td></td>
</tr>
<tr>
<td>Prolongation of PT seen in</td>
<td>33.3% of patients</td>
</tr>
<tr>
<td>Prolongation of APTT seen in</td>
<td>40.7% of patients</td>
</tr>
<tr>
<td></td>
<td>46.3% of patients</td>
</tr>
</tbody>
</table>

Table 4: Differences between our findings and those by Naithani et al

We had initially hypothesised that patients requiring more frequent transfusions have more haemolysis and thus may have a more deranged coagulation profile owing to more pronounced hypersplenism and the effect of haemolysates. However this hypothesis was not supported due to the non-significant correlation coefficients (see Table 3). It also showed that no appreciable pattern of linear correlation exists between PT, APTT and PC values and interval between transfusions (in days) and days since last transfusion.

Possible explanations for the absence of thrombotic tendency in our population might be young age and the presence of the spleen, which clears the damaged red blood cells. It may be possible that thrombotic tendency develops with increasing age, as noted by other studies, which were conducted in adults or young adults.\textsuperscript{6,7,9} Splenectomy has been implicated in the development of thrombotic state owing to the presence of more damaged red blood cells in the circulation.\textsuperscript{7,9} Other studies have noted decreases in
both thrombophilic and anti-thrombotic proteins in these patients as a consequence of liver damage; hence, the clinical picture depends on the fine balance of the contributing factors.15

Our study aimed to assess the coagulation profile of children with β-TM through investigations that are relevant in everyday clinical practice. Hence, advanced parameters of haemostasis such as platelet function, protein C and S, antithrombin-III, and D-dimers were beyond the scope of our study. These, however, may be used to elucidate the exact mechanisms of this defect, which still remains unclear.

Conclusion
It appears that a subclinical haemorrhagic tendency exists in patients with β-TM at a very early age. The intrinsic pathway appears to be more affected than the extrinsic pathway.

References

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The authors declare that they have no competing interests.

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PREVIOUS PRESENTATION