



## Subclinical haemorrhagic tendency exists in patients with $\beta$ -thalassaemia major in early childhood

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### RESEARCH

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### Abstract

#### Background

Alterations of coagulation profile have been reported in patients with  $\beta$ -thalassaemia major ( $\beta$ -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  $\beta$ -TM (mean age  $6\pm 2.5$  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 linear 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  $\beta$ -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  $\beta$ -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  $\beta$ -thalassaemia in India ranges from 3.5 to 15% in the general population, with nearly 10,000 children with  $\beta$ -thalassaemia major ( $\beta$ -TM) born every year.<sup>1,2</sup> 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  $\beta$ -TM and the presence of haemostatic abnormalities suggest the existence of a chronic hypercoagulable state.<sup>3-5</sup> These manifestations range from transient ischaemic attack, stroke, acute myocardial infarction, deep vein thrombosis to pulmonary embolism.<sup>3,6-8</sup> 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.<sup>9</sup> There have also been reports of epistaxis and haematuria, suggesting a haemorrhagic tendency in these patients.<sup>10-12</sup> 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.

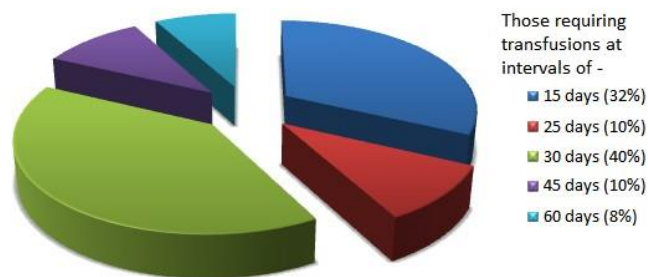
### Method

This was a cross-sectional laboratory study involving 50 paediatric patients with  $\beta$ -TM and 25 suitable controls (healthy children attending a vaccination clinic, mean age  $3.3 \pm 1.9$  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<sup>7</sup> and who were receiving chelation therapy.<sup>12</sup> Hence, we wanted to investigate this problem in a cohort of treatment-naïve 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  $150\text{--}400 \times 10^3$  per  $\mu\text{L}$ ) 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  $\alpha=0.05$ ) 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  $6 \pm 2.5$  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**

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

Mean ( $\pm$ SD) for	Patients	Controls	p value
PT (s)	14 $\pm$ 7	11 $\pm$ 1	<0.005
APTT (s)	31 $\pm$ 5	26 $\pm$ 2	<0.0001
PC ( $\times 10^3/\mu\text{L}$ )	217 $\pm$ 160	171 $\pm$ 22	>0.05

**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.

Coagulation parameter values	Percentage of patients		Percentage of controls	
	Above normal	Below normal	Above normal	Below normal
PT	12%	6%	0%	24%
APTT	6%	0%	0%	48%
PC	6%	40%	0%	12%

**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.



Correlation coefficient (r) between	PT	APTT	Platelet Count
Interval between transfusions (days)	-0.1	-0.2	0.2
Last transfusion (days back)	0.1	-0.1	0

**Table 3: Correlation between the coagulation parameters and interval between transfusions and days since transfusion for the patients**

### Discussion

In this series of paediatric patients with  $\beta$ -TM who had neither had splenectomy or chelation therapy, we noted that 40% had thrombocytopenia. The patients studied by Caocci et al were very similar to our population (no splenectomy or chelation therapy, n=25) and had normal PC.<sup>13</sup> Naithani et al, however, noted thrombocytopenia in a lower percentage (33.3%) of their paediatric patients (non-splenectomised, n=50), but these patients were already undergoing chelation therapy.<sup>12</sup> So, if hypersplenism alone were to account for thrombocytopenia in these patients, then those studied by Caocci et al would be expected to have some degree of thrombocytopenia owing to hypersplenism.<sup>13</sup> Similarly, the patients studied by Naithani et al would be expected to have even more thrombocytopenia owing to hypersplenism and chelation therapy, which can itself cause thrombocytopenia. Thus hypersplenism alone does not account for thrombocytopenia in our patients. Hence, a subclinical chronic consumption state may be responsible for the thrombocytopenia seen in our patients. This subclinical chronic consumption state could also explain the relative prolongation of PT and APTT seen in our patients compared with the controls. Although the PT, APTT and PC values for the patients in this study were mostly within the normal range, the mean values for these parameters were all raised and these changes were significant for PT (p value <0.005) and highly significant (p value <0.0001) for APTT. However, the change for PC was not significant (p value >0.05). This shows that a subclinical alteration in coagulation profile exists in polytransfused non-splenectomised paediatric patients with  $\beta$ -TM and that this alteration may be attributed to dysfunction of the coagulation cascade rather than platelet numbers. Platelet function may also have a role, but this could not be evaluated in our study.

Such significant results for PT and APTT draws our attention to another fact: that 24% of otherwise normal control subjects had PT values below the normal range, while a staggering 48% of the same controls had APTT values below the normal range. We also noted a more significant change

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 APTT 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.<sup>13,14</sup>

Table 4 shows some pertinent differences between our findings and those by Naithani et al,<sup>12</sup> 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.

Findings by	Naithani et al	This study
Thrombocytopenia seen in	33.3% of patients	40% of patients
Prolongation of PT seen in	40.7% of patients	12% of patients
Prolongation of APTT seen in	46.3% of patients	6% of patients

**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 could not be 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.<sup>6,7,9</sup> Splenectomy has been implicated in the development of thrombotic state owing to the presence of more damaged red blood cells in the circulation.<sup>7,9</sup> 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.<sup>15</sup>

Our study aimed to assess the coagulation profile of children with  $\beta$ -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  $\beta$ -TM at a very early age. The intrinsic pathway appears to be more affected than the extrinsic pathway.

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### PEER REVIEW

Not commissioned. Externally peer reviewed

### CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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### ETHICS COMMITTEE APPROVAL

Institutional Ethics Committee of Nilratan Sircar Medical College, Kolkata, India.

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