

## Role of Prothrombin time International normalized ratio and activated partial thromboplastin time in beta thalassemia major: A cross sectional study

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### Abstract

**Objectives:** To evaluate the changes in PT/ INR and APTT in Beta thalassemia major cases.

**Materials and Methods:** A cross sectional study was conducted on 100 patients diagnosed with beta thalassemia major.

**Results:** Mean PT was noted to be 15.58s and mean APTT was 41.05s. These were elevated than controls and statistically significant. Association between serum ferritin and INR was not statistically significant. Correlation graph analysis between age and APTT was positive and significant while that for ferritin and APTT was negative.

**Conclusion:** Significant alterations in PT / INR and APTT exist in beta thalassemia major patients. Additional parameters and a tailored approach is suggested.

**Keywords:** PT, APTT, INR beta thalassemia Major.

### Introduction

Thalassemia is recognized as one of the most common genetic disorder affecting the world with approximately 1-5% of the people having beta thalassemia. It is estimated that approximately 1-2 lakh individuals are born each year with severe forms of thalassemia with beta thalassemia comprising 60,000.<sup>1</sup>

The average life expectancy of patients with beta thalassemia has improved over the years as compared to that of in the previous millennium. This has led to the discovery of new set of problem such as increased hypercoagulable state in beta thalassemia in addition to the existing set of problems. Furthermore there is evidence of increased prothrombotic events, such as micro infarcts in spleen and lungs according to post mortem studies, indicating an activated coagulation pathway.<sup>2</sup>

Studies done between 1983 -1997 at Italy, observed that 4% of patients with thalassemia major and 9.6 percent of patients with thalassemia intermedia had a thromboembolic episode.<sup>3</sup>

Elevated levels of PT, APTT along with reduced levels of Protein C, Protein S and Anti-Thrombin III, have been described.<sup>4,6</sup>

### Materials and Methods

This study was a cross sectional study done on 100 known cases of beta thalassemia major attending thalassemia day care clinics between January 2017-December 2017 at KLE'S Dr. Prabhakar Kore Hospital & M.R.C. Prior to the commencement, ethical clearance for the study was obtained from the Institute ethics committee. A total of 100 BTM cases were included in the study. The objective of this study was to evaluate the abnormalities of PT INR and APTT in transfusion dependant beta thalassemia major (BTM) in patients aged above 5 years of

age. Newly diagnosed patients with beta thalassemia, haemoglobin less than 4 grams/dl, patients on warfarin or heparin, nephrotic syndrome, known cases of hemoglobinopathies other than beta thalassemia major, disseminated intravascular coagulation severe deranged liver function as assessed by history and medical records, acute thrombosis and those who lacked a minimum of 20 days gap from previous transfusion were excluded from the study. Written informed consent was obtained from the patients.

Samples for analysis were collected prior to the commencement of blood transfusion. Blood was drawn under aseptic precautions for all the tests. Two millilitre of whole blood was collected from clean venipuncture in a K2 EDTA coated vial and hemoglobin concentration, platelet count was determined using hematology auto analyser, councell 23 plus, Tulip Diagnostics.

Blood samples for determination of PT INR and APTT were collected on 3.2% Sodium Citrate vials, AcCuvet-PLUS (1:10 v/v) and platelet poor plasma was prepared for all the coagulation tests by centrifugation at 3000g for 15 min. PT was estimated by using "Uniplastin" kit supplied by Tulip Diagnostica, with an ISI of 1.1. APTT was estimated using "Liquicellin" supplied by Tulip Diagnostica. Serum ferritin estimation was done by CLIA method on Siemens Advia Centaur.

A PT of value above 13s (normal control being 13s) was taken as abnormal. APTT value of more than 30 seconds (normal control range 27s-29s) was taken as abnormal.

### Statistical Methods

Descriptive and inferential statistical analysis has been carried out in the present study. Significance is assessed at 5% level of significance. Student t test (two tailed, independent) has been used to find the significance of study

parameters on continuous scale between two groups. Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups, non-parametric setting for qualitative data analysis. Fisher exact test used when cell samples are very small.

### Statistical Software

The Statistical software namely SPSS 18.0, and R environment ver.3.2.2 were used for the analysis of the data and Microsoft Word and Excel have been used to generate graphs, tables. Pearson correlation coefficient (r) was used to test the correlation between age and APTT, Serum ferritin and APTT.

### Results

**Table 1 depicts the mean of the parameters in our study**

Parameters	Mean
PT (Control 13s)	15.61 ± 1.43
INR	1.22 ± 0.24
APTT s (Control 27-29s)	40.96 ± 6.54
Ferretin ng/ml	3801.92 ± 1585
Hemoglobin g/dl	8.38 ± 0.87
Platelet count lakhs/ mm <sup>3</sup>	2.96 ± 0.7

A total of 100 thalassemia major patients, above five years of age were included in the study, of which 42 had undergone splenectomy.

All the diagnosed cases were divided into three groups that is 5-10, 11-15 and 16 years and above. The age of the patients ranged from 5 years to 26 years.

In our study, the overall male: female ratio was 1.7: 1 with males more commonly affected than females.

Parents of 68% of thalassemic children gave a history of consanguineous marriage while parents of 28 percent of thalassemic children had non-consanguineous marriage, and 4% of parents of thalassemic children were not aware of their consanguineous status.

Prothrombin time (sec) distribution of patients studied according to age group studied, the range of PT ranged from

14-20s. Student's t- test was used to compare the PT among all the patients versus normal control. The *p*-value obtained was < 0.00001, which is strongly significant. However the variation of PT among the three groups was not significant as assessed by chi square test. (*p* = 0.107, Not Significant)

The range of APTT was between 31 to 60 seconds. The distribution of the APTT among the age groups is shown in table 2. Student's t- test was used to compare the APTT among, all the patients versus normal control. The *p*-value obtained was < 0.00001, which is strongly significant. The variation of APTT among the three groups was significant. (*p* value of 0.025, Fisher Exact test).

The hemoglobin level was reduced in all the patients. The highest hemoglobin observed was 10.3 g/dl in a patient aged 14 years and the lowest hemoglobin observed was 6.3 g/dl in a patient aged 8 years. The highest platelet count observed was 4.32 lakh /mm<sup>3</sup> in a patient aged 13 years and the lowest count observed was 1.57 lakh /mm<sup>3</sup> in a patient aged 10 years.

The serum ferritin ranged from 820 ng/ml in a patient aged 8 years to 8310 ng/ml in a patient aged 15 years. In our study we had 64% of the patients with ferritin value below 4000, while 36% of the patients had ferritin above 4000 nanograms/ml.

In the 56 number of the patients, the INR was less than 1.2 followed by 31 patients having the INR between 1.2 to 1.4 and 13 patients had the INR above 1.4 with the highest INR noted to be 1.60 in a patient aged 26 years.

Correlation analysis was done between age and APTT, and the results revealed that the pearson's correlation coefficient (r) was 0.217, with *p* value of 0.030, which was statistically significant. This implies there is a weak positive linear correlation of age with APTT. (Fig. 1)

Correlation analysis was done between serum ferritin and APTT, and the results revealed that the pearson's correlation coefficient (r) = - 0.071 and *p*= 0.482, which is not significant statistically. This implies that there is a weak linear negative correlation of ferritin with APTT and is not statistically significant. (Fig. 2) Depicts the correlation graph between APTT and serum.

**Table 2: Describes the APTT distribution among the three age groups**

APTT	Group 1	Group 2	Group 3
	5-10 yrs	11-15 yrs	16 yrs & above
31- 40s	26(47.3%)	17(73.9%)	8(36.4%)
41-50s	25(45.5%)	6(26.1%)	9(40.9%)
>51s	4(7.3%)	0(0%)	5(22.7%)
Total	55(100%)	23(100%)	22(100%)

*P*=0.025, Significant, Fisher Exact Test

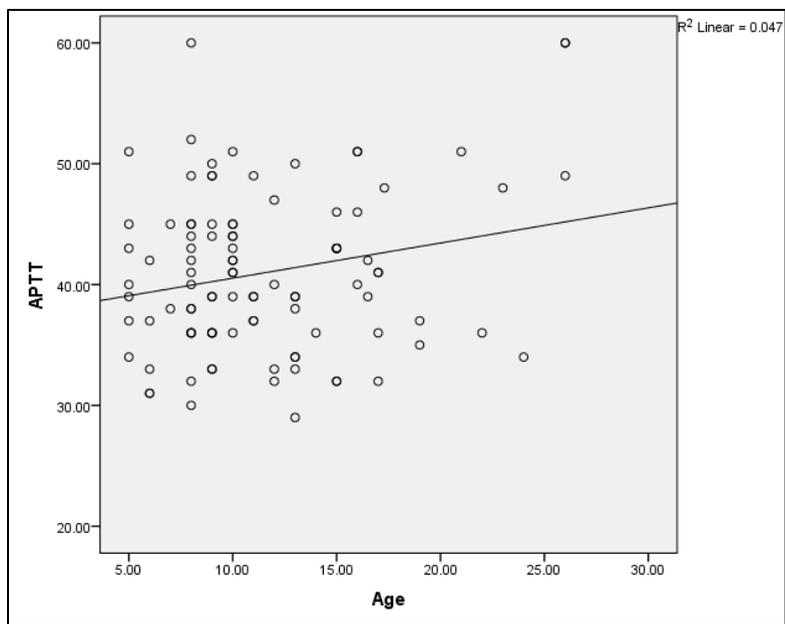


Fig. 1: Correlation graph between age and APTT

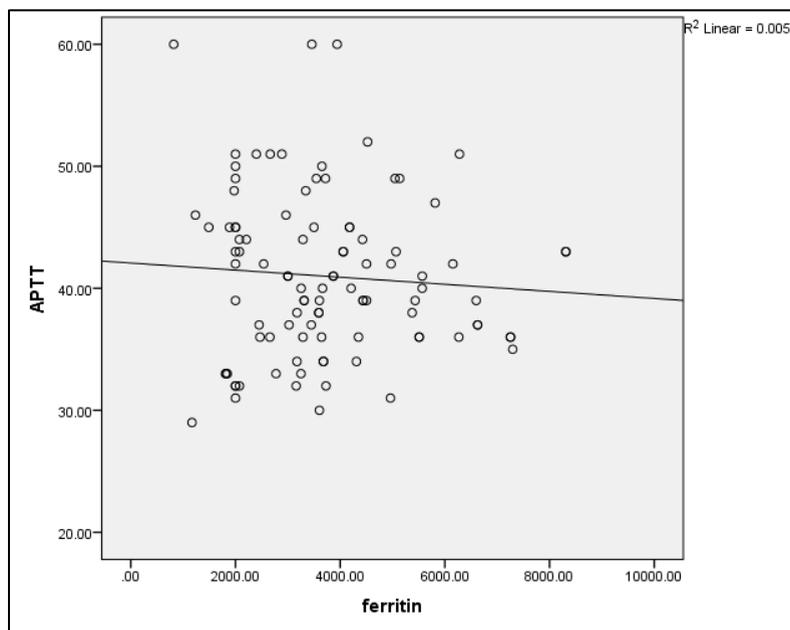


Fig. 2: Correlation graph between ferritin and APTT

**Discussion**

In our study the number of males were 63 and number of females were 37, with the overall male: female ratio of 1.7:1. According to the study conducted by Rahul Naithani, the male: female ratio of 1.5: 1 was observed.<sup>7</sup>

A positive history of consanguineous marriage among the parents of thalassemics was noted to be 68%. In a study conducted by Rakholia and Chaturvedi, the prevalence for beta thalassemia trait was noted to be 17.2 percent in and around Wardha, Maharashtra among Sindhi community.<sup>8</sup> As we noted a high degree of consanguinity among parents of thalassemia major marriage and genetic counselling should

be strongly advised for couples willing for consanguineous marriage.

The overall mean prothrombin time was noted to be 15.61 seconds which is higher than the control and statistically significant in our study. ( $p = < 0.00001$  student's t- test) These findings are in concordance with the study conducted by Rahul,<sup>7</sup> Abhishek Maithi,<sup>9</sup> and Safa A Faraj.<sup>10</sup>

The mean APTT was noted to be 40.96 seconds, which is elevated than the normal levels. This was Statistically significant ( $p = < 0.00001$  student's t- test) and in concordance with the studies done by Rahul,<sup>7</sup> Abhishek Maithi,<sup>9</sup> and Safa A Faraj.<sup>10</sup>

Ferritin level of 4000 nanograms/ml is considered as the maximum level of physiological synthesis and any levels higher than this would represent the intracellular release of ferritin, either due to inflammation or malignancy<sup>11</sup> with this background, in our study we had 64% of the patients with ferritin value below 4000, while 36% of the patients had ferritin above 4000 nanograms/ml.

The platelet count among the three groups of thalassemics, was within the normal range. A study done by Rahul Naithani<sup>7</sup> had a mean platelet count of 2.26± 1.23 lakhs/ mm<sup>3</sup> and another study conducted by Abhishek<sup>9</sup> had a platelet of 2.17± 1.60 lakhs/ mm<sup>3</sup>. Thrombocytopenia may be due to the oral chelators and also hypersplenism. In our study we did not find any cases of thrombocytopenia, this may be due to poor compliance towards serum ferritin as we have noted elevated levels of serum ferritin.

We also noted that elevated ferritin levels were associated with more prolonged INR, however, this was statistically not significant. (p=0.057, not significant, Fisher's exact test) Bleeding manifestations were seen in two patients. The first patient was aged 9 year old male with repeated episodes of haemorrhagic manifestations in the form of epistaxis which were of 1-2 episodes / month. The PT was noted to be 17s and APTT to be 49s. Splenectomy was not done and elder sibling was apparently normal.

The second patient was a 14 year old male, having undergone a splenectomy at the age of 9 years. The PT and APTT was noted to be 18s and 60s, without any major bleeding manifestations except for epistaxis which was noted since the age of 4 years, with epistaxis of 2 to 3 episodes per month. The other two siblings were apparently healthy.

In a study conducted by Rai et al<sup>12</sup> 10% of the patients had clinical hemorrhagic manifestations. Another study conducted by Ibrahim<sup>13</sup> had noticed few patients to have bleeding manifestations in the form of epistaxis. Inherited deficiency as a cause for haemorrhagic tendencies is very unlikely according to a study done by Eldor.<sup>4</sup> One might say that the synthetic function of the liver can be reduced due to secondary hemochromatosis or other chronic liver disease. We know that the liver is responsible for the clearance of ferritin from the plasma. Hence liver damage will cause increased serum ferritin concentrations due to reduced rate of removal of ferritin from the plasma. Despite the correlation between serum ferritin and both storage iron and liver damage, much of the variation in serum ferritin remained unexplained. Accurate assessment of liver iron requires analysis of biopsy samples or MRI- T2\*. Neither method was possible in this study.

The measurement of alanine aminotransferase enzyme (ALT) activity will not necessarily correlate well with ferritin release from the liver. The clearance of ferritin from the circulation is much more rapid than ALT. The biological half-life for ferritin in plasma is approximately 10 min and for alanine transaminase 6 days. Thus raised ALT levels may possibly be found some days after an episode of necrosis when ferritin levels have already fallen. Thus liver damage will cause increased serum ferritin concentrations

by reducing the rate of removal of ferritin from the plasma as well as increasing the input of ferritin, as a consequence of tissue destruction. Multivariate analysis showed that units of blood and ALT activity together only accounted for about 30% of the variation in serum ferritin concentration. The maximum rate of synthesis of ferritin is noted to be 4000ng/ml. Levels above this can strongly indicate that the excess ferritin can be due to release from tissue damage or malignancy or failure to clear by the liver.

In a study conducted by Rosnah<sup>14</sup> in the year 2014 among Malaysians, serum albumin, a reflector of synthetic function of liver, was within normal limits, despite this there were alterations in levels of Protein C and Protein S. This implies there are few mechanism, apart from liver dysfunction which can lead to abnormal hemostatic balance.

Studies done between 1983-1997 at Italy, observed that 4% of patients with thalassemia major and 9.6 percent of patients with thalassemia intermedia had a thromboembolic episode.<sup>15</sup>

A MRI imaging study done between 1996-97 at Palermo, Italy on patients with thalassemia major had 28% patients with overt stroke and 37.5% of patients with beta thalassemia minor having asymptomatic brain damage. The patients also exhibited headache and seizure.<sup>16</sup>

In a study conducted in 2006 at Mediterranean region and Iran, the authors observed that thromboembolic event occurred 4.38 times more in thalassemia intermedia than thalassemia major. They also concluded that venous thromboembolic event was more common in thalassemia intermedia and arterial thromboembolic event was common in thalassemia major.<sup>17</sup>

None of our patients had any thrombotic episodes. The reason for this could be due to regular blood transfusion and younger age group.

In a study conducted by Abhishek,<sup>9</sup> the authors found that the coagulation profile was deranged irrespective of the frequency of blood transfusion and no significant correlation existed between PT, APTT and platelet levels and the interval between transfusion and days since last transfused.

Mussumeci S, noted that both thrombophilic and anti-thrombotic proteins were reduced as a consequence of liver damage. The net clinical outcome depends on the fine balance between the pro thrombotic and antithrombotic pathways.<sup>18</sup>

Study conducted by Aruna Chhikara, found that thrombin activable fibrinolysis inhibitor (TAFI) to be elevated in beta thalassemia major patients, the higher levels of which promotes a prothrombotic episode.<sup>19</sup>

Our study aimed at assessing the coagulation profile of children with beta thalassemia major, using parameters which are routinely used. Parameters on both the procoagulant and anticoagulant pathways are better to be performed as guided by the initial results. A step wise approach or tailored approach using better indicators of coagulant and anticoagulant factors are suggested.

## Conclusion

Evaluation of PT INR and APTT is a simple test for assessing the coagulation profile in thalassemic patients. Significant alterations in PT INR and APTT exist in beta thalassemia major patients who are transfusion dependant. These alterations suggests that beta thalassemia major is a high risk condition for thrombotic or hemorrhagic tendencies. The determination of the predominant pathway should be approached on an individual basis.

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**Conflict of Interest:** None.

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