Coagulation Abnormalities in Pediatric Patients with $\beta$-Thalassemia, An experience at a Tertiary Care Hospital

Ayesha Shabbir¹, Saima Farhan², Attiq Ur Rehman¹, Neelam Mazhar¹, Sidra Hareem¹, and Iram Iqbal¹

¹Department of Hematology & Transfusion Medicine, University of Child Health Sciences, The Children's Hospital, Lahore, Pakistan
²The School of Allied Health Sciences, University of Child Health Sciences, The Children's Hospital, Lahore, Pakistan

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*Corresponding Author:
Ayesha Shabbir
Department of Hematology & Transfusion Medicine, University of Child Health Sciences, The Children's Hospital, Lahore, Pakistan
ashoo4678@gmail.com

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Abstract
Life expectancy in thalassemia has markedly improved due to consistent blood transfer and amenability with iron chelation therapy, therefore this improvement is conditioned with various thromboembolic problems of this prolonged disorder including thromboembolic complaints. Objective: To determine coagulation abnormalities in beta ($\beta$) thalassemia major patients who have been multi transfused. Methods: This observational study was conducted at Department of Haematology & Transfusion Medicine, Children hospital & University of Child Health Sciences (CH&UCHS), Lahore, from October 2022 to January 2023. The study included 60 $\beta$-thalassemic patient, age less than 16 years whose samples were compared with upper and lower normal value as regards to Prothrombin Time (PT), Active Partial Thromboplastin Time (APTT), Protein S, Protein C, liver enzymes (Alanine Transaminase (ALT), Aspartate Transaminase (AST), Gamma-Glutamyl Transferase (GGT)). The data were analyzed using IBM SPSS version 23.0. Results: When values were compared, natural clotting inhibitors (Protein S, Protein C) were remarkably reduced in $\beta$-thalassemic paed patients ($p<0.001$). PT and APTT were prolonged in thalassemic children ($p<0.05$ and $p<0.05$ correspondingly). There was substantial increase in concentrations of ALT and AST in $\beta$-thalassemic children ($p<0.05$ and $p<0.05$ correspondingly). There was substantial increase in concentrations of ALT and AST in $\beta$-thalassemic children ($p<0.05$ and $p<0.05$ correspondingly). There was substantial increase in concentrations of ALT and AST in $\beta$-thalassemic children ($p<0.05$ and $p<0.05$ correspondingly). There was substantial increase in concentrations of ALT and AST in $\beta$-thalassemic children ($p<0.05$ and $p<0.05$ correspondingly). There was substantial increase in concentrations of ALT and AST in $\beta$-thalassemic children ($p<0.05$ and $p<0.05$ correspondingly). 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Conclusions: Marked changes in coagulation inhibition supporting thrombotic tendency was observed in thalassemic children. There were reduced levels of protein C and protein S independent of slightly prolonged PT and APTT and elevated levels of ALT, AST with normal GGT in thalassemic children.

Introduction
Thalassemia is inherited hemolytic condition initiated due to partial or complete defect of $\alpha$ or $\beta$ globin chain production [1]. Basically, thalassemia is a heterogeneous group of genetic heritable disorders of hemoglobin synthesis, which is considered to be the most monogenic condition in the world [2]. It is a life-threatening situation which poses severe health and financial strains on patients and their families [3]. Almost 70,000 children with different types of thalassemia are born every year [4]. Clinically three main classes are transfusing ion dependent thalassemia major, non-transfusion dependent thalassemia intermedia with a moderate degree of anemia due to variety of genetic defects and thalassemia minor which is usually due to a carrier state for alpha or beta thalassemia and characterized by erythrocyte microcytosis and mild or no anemia [5]. $\beta$-thalassemia major is most common form. It results from mutations within beta ($\beta$) globin gene which effect $\beta$-globin chain production. $\beta$-thalassemia includes three kinds: Thalassemia Major (also called Cooley or Mediterranean Anemia), Thalassemia Intermedia and Thalassemia Minor also called $\beta$-thalassemia carrier or heterozygous $\beta$-
Thalassemia patients already have a chronic subclinical hypercoagulable state. Hypercoagulability in thalassemic patients is understandable, it depends on different variables like closed renal and pulmonary vessels, high numbers of thromboembolic incidents, early atherosclerosis, compromised platelet accumulation, reduced platelet survival, and high urinary metabolites of thromboxane A2 (TXA2) and prostacyclin (PGI2) [7]. Red blood cell membrane abnormality, oxidative damage caused by free hemoglobin, lack of several proteins of clotting cascade, increased leukocyte activation are various factors that contribute to the disease' etiopathogenesis [8]. These problems should be a part of routine treatment procedures and follow up must be planned keeping in view these issues [9]. Different Researches shows that hypercoagulable state along with thromboembolic events in young patients but results were debatable and not comprehensive [10]. These patients are dependent on blood transfusion throughout their lives [1]. As prognosis is improving in these patients, degree of various severe problems is increasing too, TEE are one of such serious issues. Prolongation of PT and APTT, low level of natural anticoagulants like protein C, protein S have been labelled in these patients, though the procedures implying in thrombotic affinity observed in some patients and hemorrhagic indicators have not been completely illuminated [11]. Limited literature is available on coagulation defects in paed thalassemic patient, so this study is planned for the up gradation of knowledge to get them managed early to prevent complications. In this study we aimed to determine coagulation abnormalities in β-thalassemia patients who have been multi transfused.

Methods

This observational study was conducted at Department of Haematology and Transfusion Medicine, Children Hospital and University of Child Health Sciences (CH&UCHS), Lahore from October 2022 to January 2023. The participants of this study included 60 children with β-thalassemia. The sample size was calculated from formula: Here,

\[ n = \left( \frac{Z^2 \alpha}{2 \beta^2} \right) \left[ \frac{p(1-p)}{\Delta^2} \right] \]

Multiple transfused thalassemia major patients with age <16 year were included in this study. The samples were properly labelled and collected in citrate vial and clot activator gel vial. Consecutive sampling method was used for the study. While children with preceding or current thromboembolic events, family history of clotting disorder, using anticoagulant and with any indication of liver failure or cardiomyopathy were excluded from the study. After acquiring informed consent, the blood sample was collected from multiple transfused thalassemia major patients in 3.2 % sodium citrate (blue top) vial for performing coagulation tests and in gel vial (yellow top) for performing liver function tests. Venous blood collected in citrate vial was centrifuged and plasma was separated. Plasma was examined for Protein C, Protein S, PT and APTT in STA Compact Analyzer. Venous blood collected in gel vial was centrifuged and serum was separated. Liver function tests (AST, ALT, and GGT) was performed on serum in AU 480 Chemistry Analyzer. The results of β-thalassemic patients obtained were compared with upper normal values of variables. The data was entered and evaluated using IBM-SPSS V-23. Continuous variables such as age, PT, APTT, Protein C, Protein S, ALT, AST, GGT were described as Mean±SD, however categorical variables like gender was in form of frequencies and percentages. One sample t-test was applied to compare the means of upper or lower normal value and cases. A p-value of < 0.05 was considered statistically significant. Ethical approval was acquired from ethical committee of The School of Allied Health Sciences, University of Child Health Sciences, Lahore.

Results

A total of 60 β-thalassemic children participated in the study. This study was completed in a duration of about 4 months (From October 2022 to January 2023) in the Department of Hematology and Transfusion, Children Hospital, Lahore. Baseline demographic features are summarized in Table 1.

Table 1: Baseline Demographics of subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>Category</th>
<th>N (%)</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Year)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
<td>32(53.3)</td>
<td>7.94±3.36</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>28(46.7)</td>
<td></td>
</tr>
</tbody>
</table>

SD: Standard Deviation. Categorical Variables are in form of N(%), while continuous variable is in form of Mean±SD. The values of clotting inhibitors (protein S and protein C), and Liver Functional Enzymes is represented in Table 2. Considerably reduction in protein C and protein S were evident in thalassemic children. PT and APTT were slightly prolonged in thalassemic patients. The mean levels of ALT and AST were significantly higher but the mean level of GGT was not raised in thalassemic children.

Table 2: One sample t-test Analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Upper/Lower Normal Value</th>
<th>Mean ± SD (Cases)</th>
<th>t-value</th>
<th>p-value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>14</td>
<td>14.29±2.17</td>
<td>1.034</td>
<td>0.305</td>
<td>0.27-0.85</td>
</tr>
<tr>
<td>APTT</td>
<td>34</td>
<td>36.30±6.45</td>
<td>3.15</td>
<td>&lt;0.05</td>
<td>0.96-6.30</td>
</tr>
<tr>
<td>ALT</td>
<td>42</td>
<td>86.12±59.20</td>
<td>5.773</td>
<td>&lt;0.001</td>
<td>28.82-59.41</td>
</tr>
<tr>
<td>AST</td>
<td>37</td>
<td>81.86±58.36</td>
<td>5.953</td>
<td>&lt;0.001</td>
<td>29.77-58.83</td>
</tr>
<tr>
<td>GGT</td>
<td>30</td>
<td>24.96±10.34</td>
<td>3.782</td>
<td>&lt;0.001</td>
<td>7.72-2.38</td>
</tr>
<tr>
<td>Protein C</td>
<td>79</td>
<td>28.83±8.44</td>
<td>47.066</td>
<td>&lt;0.001</td>
<td>52.35-47.99</td>
</tr>
<tr>
<td>Protein S</td>
<td>65</td>
<td>41.08±10.89</td>
<td>17.005</td>
<td>&lt;0.001</td>
<td>26.73-21.10</td>
</tr>
</tbody>
</table>
The shielding effect in keeping the concentration of one of the indicators of oxidative stress because of its GGT is present on plasma membranes and it is considered decreased in studies conducted by Esra Emaad Jabbar leads to elevated serum levels of ALT and AST [14]. In a thalassemia patients [16]. Acute or Chronic Liver damage is the major danger factors for the Vitamin D deficiency in high iron deposition in body along with elevated AST were enzyme [15]. Some studies have shown that moderate to dissolution of tissues that results in leaking of ALT and AST in the blood circulation therefore increasing the action of enzyme [16]. Some studies have shown that moderate to high iron deposition in body along with elevated AST were the major danger factors for the Vitamin D deficiency in β-thalassemia patients [16]. Acute or Chronic Liver damage leads to elevated serum levels of ALT and AST [14]. In a study conducted by Esra Emaad Jabbar et al., GGT level was decreased in β-thalassemic patients same as in our study. GGT is present on plasma membranes and it is considered one of the indicator of oxidative stress because of its shielding effect in keeping the concentration of glutathione (GSH). It functions to breakdown it exterior to cell, so production of free radicals can result in intracellular glutathione reduction and therefore increased GGT level in circulation [17, 18]. The level of GGT was not elevated in these patients in our study and this can be explained in terms that there are different stages of liver cirrhosis and their liver might be precirrhotic. PT (p<0.305) and APTT (p<0.05) were prolonged. The borderline elongation of PT and APTT in our study are reduced than previous studies. Our findings are consistent with those found in other studies conducted by Naithani et al [3], Srevatsa et al., [19] and Faraj [20]. Prolonged PT and APTT may be caused by parenchymatous liver damage due to iron over load caused by multiple transfusions.

**CONCLUSIONS**

Marked changes in coagulation inhibition supporting thrombotic tendency was observed in thalassemia children. There was reduction in protein C and S levels, prolongation of PT and APTT and elevated levels of liver enzymes in thalassemia children due to deranged liver functions.

**Authors Contribution**

Conceptualization: SF
Methodology: AUR, NM
Formal Analysis: AS
Writing-review and editing: AS, AUR, SH, II

All authors have read and agreed to the published version of the manuscript.

**Conflicts of Interest**

The authors declare no conflict of interest.

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**References**


