Evaluation of Protein S and Protein C Levels in B-Thalassemic Patients and Its Association with Blood Transfusion

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Abstract:

Background: Thalassemia is a chronic hemolytic anemia due to a partial or complete deficiency in the synthesis of α-globin chains or β-globin chains that compose the major adult hemoglobin (α 2β 2). Protein C is a vitamin K-dependent serine protease and naturally occurring anticoagulant that plays a role in the regulation of hemostasis by inactivating factors Va and VIIIa in the coagulation cascade. Protein S is a non-enzymatic cofactor for activated protein C–mediated inactivation of factor VIIIa and Va. In addition, protein S may have activated protein C–independent anticoagulant activity. Aim of study: To evaluate protein S&C levels in thalassemic patients & its relation to blood transfusion.

Methods: The study included 80 subjects divided into two groups: Case group included 60 patients who were a known cases of transfusion dependent B-thalassemia and control group included 20 participants who were healthy and matched in age and gender with case group. Patients with other forms of hemolytic anemia like sickle thalassemia, who suffering from severe hepatic or cardiac dysfunction, those with other hemoglobinopathies, were excluded from the study. Plasma protein S and C Ag were calculated in both patient groups and in control group by ELISA.

Results: Plasma protein C was significantly lower in patients with splenectomy in comparison with patients without splenectomy. Protein C and S were significantly lower in patients with non-frequent than in patients with frequent blood transfusion. There was significant positive correlation between plasma protein C and protein S levels.

Conclusion: Patients with thalassemia major have lower level of protein C and protein S than non-thalassemic patient. Frequency of blood transfusion can affect level of protein C & protein S. Splenectomized patients have lower levels of protein C and S than non-splenectomized.

Introduction:

Thalassemia is a chronic hemolytic anemia due to a partial or complete deficiency in the synthesis of α-globin chains or β-globin chains that compose the major adult hemoglobin (α 2β 2). (1) It is caused by one or more mutations in the corresponding genes. The unpaired globin chains are unstable; they precipitate intracellularly, resulting in hemolysis, premature destruction of red blood cell [RBC] precursors in the bone marrow, and a short life-span of mature RBCs in the circulation. (13)

Beta-Thalassaemia is one of the commonest genetic disorders in the world. It occurs due to mutation in β-gene of autosomal chromosome11. (2) It is divided into three categories based on the zygosity of the beta-gene mutation, Beta-thalassemia minor, Beta-thalassemia major and beta-thalassemia intermedia (3).

Protein C is a vitamin K-dependent serine protease and naturally occurring anticoagulant that plays a role in the regulation of hemostasis by inactivating factors Va and VIIIa in the coagulation cascade. (4)

Protein S is a non-enzymatic cofactor for activated protein C–mediated inactivation of factor VIIIa and Va. In addition, protein S may have activated protein C–independent anticoagulant activity by directly binding and inhibiting factor VIIIa in tenase complex, factor Va and factor Xa in the prothrombin's complex. (5)

Several etiologic factors may play a role in the pathogenesis of the hypercoagulable state in thalassemia such as platelet activation and increased circulating aggregates, shortened platelet survival, increased urinary excretion of thromboxane A2 and prostacyclin metabolites, decreased levels of naturally occurring anticoagulants such as protein C and protein S, and an elevated plasma level of thrombin-antithrombin complex have also been reported. (5)
Patients, Materials and Methods:

It is a case control study, samples from 80 subjects aged between 2 – 19 years and divided into two groups: 1- Case group: Included 60 patients who were known cases of transfusion dependent B-thalassemia. The patients subclassified according to frequency of blood transfusion into frequent transfusion (37 patients) and non-frequent transfusion (23 patients), and also subclassified according to spleen status into splenectomized (11 patients) and non-splenectomized (49 patients). 2- Control group: Included 20 participants who were healthy and matched in age and gender with case group.

Four ml of blood were withdrawn by venipuncture under aseptic technique from every patient (before receiving the blood transfusion) and every control. The total venous blood sample divided into two smaller samples as follows: 1- Two ml in EDTA tube for complete blood count, blood film and retic count. 2- (1.8 ml) of blood in (0.2 ml) of trisodiumcitrate with gentle mixing. Plasma was obtained by centrifugation of blood at room temperature for 15 minutes at 1000 g within 30 minutes of collection, plasma divided into aliquots then stored at (-80°C) which is then used to measure protein S & protein C antigen by ELISA. Normal pooled plasma was prepared by pooling of plasma from 20 normal healthy donors and then divided into aliquots each containing about 1ml stored at (-80°C).

Inclusion criteria & Exclusion criteria

The Inclusion criteria of patients are those with transfusion dependent B-thalassemia, Not receiving blood transfusion in the last 2 week.

The Exclusion criteria included the Patients suffering from severe hepatic or cardiac dysfunction; Patient with history of familial thrombophilia or use of anticoagulant therapy or anti-platelet drugs.

Statistical analysis

Data were summarized, analyzed and presented using statistical package for social sciences (SPSS) version 23 and Microsoft Office Excel 2010. Quantitative variables were expressed as mean and standard deviation (SD); whereas, categorical variables were expressed as number and percentage. Independent samples t-test was used to compare mean values between two groups, while Chi-square test was used to study associations between any two categorical variables; however, Yates correction was used instead when more than 20 % of cells have expected count less than 5 and Spearman correlation was used to assess correlation between quantitative variables. The level of significance was set at P value ≤ 0.05.

The results:

Hematological characteristics of thalassemia patients and control subjects are shown in table 1. The mean RBC, Hb, PCV and MCV were significantly lower in patients with thalassemia than control groups. However, the mean RDW was significantly higher in patients with thalassemia than control groups as shown in table 1.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients group, n = 60</th>
<th>Control group, n = 20</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td>60.43 ±2.99</td>
<td>38.4 ±4.70</td>
<td>† 0.001&gt;</td>
</tr>
<tr>
<td>Hb g/dl</td>
<td>87.66 ±7.66</td>
<td>12.11 ±12.74</td>
<td>† 0.001&gt;</td>
</tr>
<tr>
<td>% PCV</td>
<td>281 ±24.06</td>
<td>359 ±38.80</td>
<td>† 0.001&gt;</td>
</tr>
<tr>
<td>MCV fl</td>
<td>382 ±78.87</td>
<td>654 ±81.99</td>
<td>† 0.011</td>
</tr>
<tr>
<td>% RDW</td>
<td>431 ±3.23</td>
<td>445 ±15.65</td>
<td>† 0.001&gt;</td>
</tr>
<tr>
<td>Serum Ferritin</td>
<td>1298.56 ±3164.20</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

**n:** number of cases; **SD:** standard deviation; †: independent samples t-test; **HS:** Highly significant at P ≤ 0.01
As shown in table (2):
Mean plasma protein C of thalassemia patients was 70.71 ±23.72 and that of control group was 77.88 ±6.24; therefore, protein C was lower in thalassemia patients in comparison with control group; however, the difference was statistically insignificant (P = 0.187), as shown in table (2).

Mean plasma protein S of thalassemia patients was 74.24 ±19.50 and that of control group was 81.58 ±8.24; therefore, protein S was lower in thalassemia patients in comparison with control group; however, the difference was statistically insignificant (P = 0.107), as shown in table (2).

Table 2: Comparison of plasma protein C and protein S between thalassemia patients and control subjects

<table>
<thead>
<tr>
<th>Protein</th>
<th>Control group</th>
<th>Thalassemia group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 20</td>
<td>n = 60</td>
<td></td>
</tr>
<tr>
<td>Protein C</td>
<td></td>
<td></td>
<td>† 0.187</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>±6.24 77.88</td>
<td>±23.72 70.71</td>
<td>NS</td>
</tr>
<tr>
<td>Range</td>
<td>-91.30 60.19</td>
<td>24.01-150.00</td>
<td></td>
</tr>
<tr>
<td>Protein S</td>
<td></td>
<td></td>
<td>† 0.107</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>±8.24 81.58</td>
<td>±19.50 74.24</td>
<td>NS</td>
</tr>
<tr>
<td>Range</td>
<td>-98.00 66.00</td>
<td>-128.00 38.00</td>
<td></td>
</tr>
</tbody>
</table>

Mean plasma protein C was significantly higher in patients with frequent blood transfusion than in patients with non-frequent blood transfusion (P < 0.001), 84.35 ±17.26 versus 48.77 ±14.33, respectively, as shown in table (3). In addition, it was significantly higher in control subjects than in patients with non-frequent blood transfusion (P < 0.001), 77.88 ±6.24 versus 48.77 ±14.33, respectively, as shown in table (3), but, there was no significant difference between control subjects and patients with frequent blood transfusion (P = 0.110), 77.88 ±6.24 versus 84.35 ±17.26, respectively, as shown in table (3).

Besides, Mean plasma protein S was significantly higher in patients with frequent blood transfusion than in patients with non-frequent blood transfusion (P < 0.001), 84.64 ±16.54 versus 57.50 ±9.87, respectively, as shown in table (3). In addition, it was significantly higher in control subjects than in patients with non-frequent blood transfusion (P < 0.001), 81.58 ±8.24 versus 57.50 ±9.87, respectively, as shown in table (3), but, there was no significant difference between control subjects and patients with frequent blood transfusion (P = 0.404), 81.58 ±8.24 versus 84.64 ±16.54, respectively, as shown in table (3).

Table 3: Comparison of plasma protein C and S between patients with frequent blood transfusion and patients with non-frequent blood transfusion

<table>
<thead>
<tr>
<th>Protein</th>
<th>Control</th>
<th>Blood transfusion</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 20</td>
<td>Frequent n = 37</td>
<td>Non-frequent n = 23</td>
</tr>
<tr>
<td>Protein C</td>
<td>±6.24 77.88</td>
<td>84.35 ±17.26</td>
<td>±14.33 48.77</td>
</tr>
<tr>
<td>P -value</td>
<td>0.110</td>
<td>0.001 &gt;</td>
<td>0.001 &gt;</td>
</tr>
<tr>
<td>Protein S</td>
<td>±8.24 81.58</td>
<td>±16.54 84.64</td>
<td>±9.87 57.50</td>
</tr>
<tr>
<td>P -value</td>
<td>0.404</td>
<td>0.001 &gt;</td>
<td>0.001 &gt;</td>
</tr>
</tbody>
</table>

Iraqi Journal of Cancer and Medical Genetics (IJCMG)
Volume 13 - Number 1 - 2020
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Discussion:

In the current study, mean plasma protein C of thalassemic patients was non-significantly lower in comparison with control. Also mean plasma protein S of thalassemic patients was non-significantly lower in comparison with control group. In contrary, different results observed in Hassan et al study at 2010, in which plasma levels of protein C and protein S were significantly lower in thalassemic patients than in the control group. Also they concluded that protein C plays a major role in the hypercoagulable state in thalassemic patients and necessary to raise the issue as to whether it would be cost-beneficial to recommend prophylactic antithrombotic therapy in high-risk thalassemic patients.

In the same consistence, Rosnah and colleagues in their study that conducted in Malaysia in 2014, Their result showed that mean Protein C and protein S levels were significantly lower in thalasemia patients compared to age-matched normal control, concluded a significantly decreased Protein C and protein S in thalasemia patients which might suggests hypercoagulable state in thalasemia patients.

Furthermore, Study by Abosdera and colleagues in 2014 on 50 β thalasemia major patients revealed different findings in the form of significantly low level of Protein C and protein S among thalassemic patients in comparison to age- and sex-matched healthy controls included in the study, they concluded that significant changes in natural coagulation inhibition and fibrinolysis processes favoring thromboembolism can be detected in otherwise healthy thalassemic children.

Different sample size contributed in each study can determined significance association, additionally, the cause behind the reduction of these factors can be attributed to fact that these proteins are very sensitive to even a mild degree of impairment of the synthetic function of the liver that not to be yet diagnosed, which is a common occurrence in thalassemia due to various causes as hepatic hemosiderosis, viral infections, and vitamin and protein deficiency, low Protein C, protein S level can be due to inherited deficiency of Protein C and protein S, in addition to increase in consumption. However, it is very unlikely because even the prevalence of more common congenital thrombophilic mutations like factor V Leiden and prothrombin G20210A mutations are not increase in thalassemia Patient.

Additionally, protein C was significantly lower in patients with splenectomy in comparison with patients without splenectomy, while protein S was non-significantly reduced between patients with splenectomy than those without splenectomy. In comparison to other studies, an Egyptian study done by Hassan and colleagues in 2010, in which Fifty patients with β-thalassemia major (30 non-splenectomized and 20 splenectomized) and 20 healthy children as a control group were included, results obtained showed that Plasma levels of protein C and protein S were significantly lower in splenectomized patients in comparison to those in control group.

Different sample size can have considered as one of the causes of the difference between the studies. The greater reduction in protein C and S levels in splenectomized thalassemic patients might be due to procoagulant on the surface of RBCs and abnormal platelets that are not removed from circulation in the case of splenectomy, with the resultant consumption of protein C and protein S in an attempt to control the hypercoagulable state.

In this study, mean protein C was significantly lower in patients with non-frequent blood transfusion than those with frequent transfusion. Besides, protein S also was significantly lower in patients with non-frequent blood transfusion.

In comparison to other studies, an Egyptian study done by Hassan and colleagues in 2010, found that thalassemic patients with frequent blood transfusions, whether splenectomized or not, had higher levels of protein C than their peers with infrequent blood transfusion (80.8±3.8 vs. 70.2±10.4, P=0.05), while protein S was non-significantly decreased in those had non-frequent transfusion than those with frequent transfusion (81.3±2.5 vs. 71±8.5, P=0.1) (6). In Rosnah et al study in 2014, results showed a significantly lower mean of Protein C in splenectomized patients [54.00(14.36)] and non-splenectomized [54.84 (12.86)] than control [94.14 (16.34)]. However, the difference between splenectomized and non-splenectomized was not statistically significant. The impact of frequency of blood transfusion on the hypercoagulable state in Eldor et al study in 2002, found that levels of protein C and protein S were lower in infrequently transfused patients than in frequently transfused ones (14,12)

Conclusion:

Patients with thalassemia major have lower level of protein C and protein S than non-thalassemic patient. Frequency of blood transfusion can affect level of protein C and protein S & Splenectomized patients have lower levels of protein C and S than non-splenectomized.
References: