Plasma substance P and soluble P-selectin as biomarkers of β-thalassemia induced hypercoagulability

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KEYWORDS
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Tachykinins;
P-selectin

Abstract Background: Hypercoagulability in thalassemia especially in thalassemia major has emerged as a complication of the disease. There is evidence of increased platelet aggregation and increased proportion of platelets expressing P-selectin in thalassemia. P-selectin is a cell adhesion molecule which plays a key role in hemostasis and thrombosis, mediating platelet rolling and generating procoagulant molecules. Substance P is one of the tachykinins which constitute a family of neuropeptides. It now appears that platelets contain substance P which is released upon stimulation leading to faster and more extensive aggregation.

Objective: To detect the possible role of substance P and soluble P-selectin (sP-selectin) as biomarkers of hypercoagulability in patients with beta-thalassemia major.

Subjects and methods: Venous blood samples were collected from ten normal control subjects and thirty patients with beta-thalassemia major (divided into two groups, splenectomized and unsplenectomized). To all studied individuals, plasma substance P and sP-selectin were assayed by an enzyme linked Immunosorbent assay (ELISA).
1. Introduction

Beta-thalassemia is an inherited autosomal recessive hematologic disorder that causes hemolytic anemia because of the decreased or absent synthesis of the beta-globin chain. In Egypt, it was estimated that 1000/1.5 million per year live births will suffer from thalassemia. Beta-thalassemia is the most common type with a carrier rate ranging from 5.3% to 9% and a gene frequency of 0.03%.  

Beta-thalassemia is clinically classified into: thalassemia major, intermedia and minor depending on the demand for blood transfusion and pathophysiology. The mainstay of managing these patients relies on blood transfusion and chelation. A state of hypercoagulability has been recognized in beta-thalassemia but the mechanisms underlying it are still unsettled.

Platelet activation has been incriminated in starting the hypercoagulable state. Enhanced thrombin generation activates the platelets, monocytes, granulocytes and endothelial cells which further enhance the prothrombotic process. There is evidence of increased platelet aggregation and increased proportion of platelets expressing P-selectin in thalassemia. P-selectin (CD62P) is a member of the selectin family of cell adhesion receptors, which mediate binding to specific carbohydrate ligands. P-selectin is stored in alpha-granules of platelets and Weibel–Palade bodies of endothelial cells. Upon cellular activation, P-selectin translocates with the granule to the cell surface, where it mediates adhesion to leukocytes or endothelium.

Substance P is a member of the tachykinin family of peptides and has clinically been considered a neuropeptide. Its interaction with tachykinin receptors on vascular endothelial or smooth muscle cells can cause vasodilation or contraction, respectively. It now appears that platelets contain substance P which is released upon stimulation leading to faster and more extensive aggregation.

The aim of the present work was to investigate the role of plasma substance P and soluble P-selectin as biomarkers of beta-thalassemia induced hypercoagulability.

2. Subjects and methods

Thirty patients with beta-thalassemia major followed up at the Hematology Department, Medical Research Institute were enrolled. A written consent for participating in the study was taken according to the declaration of Helsinki and approved by the ethics committee of the Medical Research Institute. Because splenectomy is a well known risk factor for thrombosis in thalassemia patients, they were divided into two groups:

2.1. Group I

Fifteen unsplenectomized patients, their mean age was (22.37 ± 8.96) years.

2.2. Group II

Fifteen splenectomized patients, their mean age was (20.28 ± 7.53) years.

Ten age and sex matched healthy individuals served as normal control group. Venous blood sample was withdrawn from control subjects and from patients after an overnight fasting and just prior to blood transfusion. To all studied individuals, a full history taking and clinical examination were done. Laboratory investigations included: complete blood picture, serum ferritin and liver and kidney function tests. The plasma levels of circulating substance P and sP-selectin were assayed by ELISA (R&D Systems, USA).

All patients received blood transfusion at a rate of one unit packed red blood cells every fortnight. They were chelated by desferrioxamine and the oral chelator deferipone. Most of them were infected with hepatitis C virus.

2.3. Statistical analyses

Statistical analyses were conducted using the statistical software package of SPSS version 11.5 (SPSS Inc, Chicago, USA). Differences between groups were assessed by the Mann Whitney U test for nonparametric variables. Pearson’s correlation coefficients were calculated to evaluate the association between relevant parameters. Statistical significance was set at \( p < 0.05 \).

3. Results

As can be seen in (Table 1), there was no statistically significant difference in white blood cell count (WBC) among the studied groups and controls (\( P = 0.97 \) and \( P = 0.46 \) respectively). No statistically significant difference was found in hemoglobin concentration on comparing both groups. A significantly higher normoblast count (/100WBC) was found in the splenectomized group compared to the unsplenectomized group (\( P = 0.001 \)). The mean platelet volume (MPV) did not differ among the studied groups. The median value of platelet count in the splenectomized group was significantly higher than that in control and unsplenectomized groups (\( P = 0.001 \) and \( P = 0.000 \) respectively). Furthermore, splenectomized group had a higher serum ferritin level compared to the unsplenectomized group (\( P = 0.021 \)). Also it was higher in patient groups versus the controls (\( P = 0.000 \)).

Results: Higher levels of plasma substance P and sP-selectin were observed in thalassemic patients versus the controls. Both substance P and sP-selectin were significantly higher in the splenectomized group of patients.

Conclusions: Substance P and sP-selectin might have a role in platelet activation and subsequent hypercoagulability in thalassemic patients.

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With respect to substance P, higher levels were observed in thalassemic patients versus the controls (P = 0.000) especially in the splenectomized group (P = 0.009) but no significant difference was found between both patient groups. Regarding sP-selectin, it was significantly higher in the patient groups compared to the control (P = 0.037) and especially in the splenectomized group compared to unsplenectomized group and the controls (P = 0.016 and P = 0.003 respectively) (Table 2).

A significant positive correlation was found between sP-selectin and plasma substance P (P = 0.000), WBCs count (P = 0.025), normoblasts (P = 0.040) and MCV (P = 0.022). Additionally, a borderline significance was found with serum ferritin (P = 0.053) while there was no significant correlation with platelet count (P = 0.584) or MPV (P = 0.867) (Table 3).

With respect to substance P, a significant negative correlation was observed between plasma substance P and MPV (P = 0.034). On the other hand, there was no significant correlation between plasma substance P and WBC count (P = 0.073), normoblasts (P = 0.503), MCV (P = 0.083), platelet count (P = 0.123) or serum ferritin (P = 0.257) in all patients with β-thalassemia major (Table 3).

4. Discussion

A profound hemostatic derangement has been observed in patients with thalassemia4, yet the mechanism underlying this hypercoagulable state is multifactorial12-18. Platelet activation has been incriminated. It is caused by the scavenging of endothelial-derived nitric oxide (NO) which causes vasoconstriction and decreased blood flow.10 Thrombocytosis and spontaneous platelet activation and aggregation, especially after splenectomy remains an attractive pathophysiologic issue.20 This has been observed in the present study where thrombocytosis was significant in splenectomized patients which by itself predisposes them to thrombosis.

In the present study, a significant increase in sP-selectin was observed in thalassemic patients especially in the splenectomized group reflecting a state of endothelial activation and damage which further enhances platelet activation. This is in agreement with Kanawaki et al.21 and Krishman et al.22 In addition, Kato et al.18 showed increased plasma levels of vascular cell adhesion molecules (sVC-AMs), sE-selectin, sP-selectin in sickle cell patients compared to healthy individuals. In addition, Hayashi et al.23 reported that P-selectin plays a key role in immune system mediated inflammation through promoting the adherence of leukocytes to activated platelets and endothelium, leukocyte migration, cytokine release and secretion of growth factors at the site of injury. Furthermore, many authors highlighted the role of sP-selectin in arterial thrombogenesis by forming large stable platelet leukocyte aggregates.24,25 Our findings are also supported by Sullivan et al.26 and Smyth et al.27 who demonstrated that venous thrombosis was decreased in P-selectin deficient mice.

**Table 1** Hematological results in all studied groups.

<table>
<thead>
<tr>
<th></th>
<th>Control group (n = 10)</th>
<th>Unsplenectomized group (n = 15)</th>
<th>Splenectomized group (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WBC (×10^3/L)</strong></td>
<td>Range: 5–8 Median: 6.5</td>
<td>4.2–10</td>
<td>3.3–46.8</td>
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<tr>
<td></td>
<td>P: 0.97</td>
<td>6.0</td>
<td>7.6</td>
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<td></td>
<td></td>
<td>0.97</td>
<td>0.46</td>
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<td></td>
<td></td>
<td>0.49</td>
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<tr>
<td><strong>Hb (g/dl)</strong></td>
<td>Range: 11.6–14.9</td>
<td>6.4–10.5</td>
<td>7.1–10.9</td>
</tr>
<tr>
<td></td>
<td>Median: 12.65</td>
<td>8.75</td>
<td>8.2</td>
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<td></td>
<td></td>
<td>0.000†</td>
<td>0.000†</td>
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<td></td>
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<td>0.093</td>
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<tr>
<td><strong>Normoblast count/100 WBC</strong></td>
<td>Range: 0–0 Median: 0</td>
<td>0–80</td>
<td>0–460</td>
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<tr>
<td></td>
<td></td>
<td>20</td>
<td>31</td>
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<tr>
<td></td>
<td></td>
<td>0.006†</td>
<td>0.001*</td>
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<td>0.001*</td>
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<tr>
<td><strong>Platelets (×10^9/L)</strong></td>
<td>Range: 217–402 Median: 253.5</td>
<td>115–458</td>
<td>251–629</td>
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<tr>
<td></td>
<td></td>
<td>232.5</td>
<td>378</td>
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<td></td>
<td></td>
<td>0.413</td>
<td>0.000†</td>
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<td>0.000†</td>
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<tr>
<td><strong>MPV</strong></td>
<td>Range: 8.6–10.9</td>
<td>7.9–10.7</td>
<td>8.1–10.3</td>
</tr>
<tr>
<td></td>
<td>Median: 9.15</td>
<td>9.25</td>
<td>9.2</td>
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<td></td>
<td></td>
<td>0.731</td>
<td>0.820</td>
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<td></td>
<td></td>
<td>0.971</td>
<td></td>
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<tr>
<td><strong>Ferritin (ng/ml)</strong></td>
<td>Range: 41–90 Median: 58.5</td>
<td>804–1400</td>
<td>1070–8700</td>
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<tr>
<td></td>
<td></td>
<td>1356.5</td>
<td>3200</td>
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<td></td>
<td>0.000†</td>
<td>0.000†</td>
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<td></td>
<td></td>
<td>0.021†</td>
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Significance was considered at level of P < 0.05.
* Significantly different from control group.
§ Significantly different from unsplenectomized group.
In the present study, a positive correlation was found between sP-selectin, a marker of platelet activation, and normoblast count reflecting the relation between platelet activation and the subsequent prothrombotic state and normoblasts. This agrees with Garozzo and his colleagues who explained the hypercoagulable status of thalassemic patients by the fact that their normoblasts carried intercellular adhesion molecule-2 (ICAM-2) and platelet endothelial cell adhesion molecule-1 (PECAM-1).

Moreover, the role of red cell size in the pathogenesis of hypercoagulability has been studied by Atichartakarn and his colleagues. They reported that smaller sized RBCs are more thrombogenic especially after splenectomy. Their data supported by Warefeld and Myers who reported that thrombosis and inflammation are inter-related.

Regarding substance P, to our knowledge it has not been studied in patients with thalassemia. In the present study, significantly elevated levels of substance P were observed in patients with thalassemia compared to the control and the elevated levels were more prominent in splenectomized patients reflecting a higher level of platelet activation.

In the present study, we found a significant positive correlation between substance P and sP-selectin, reflecting the role of substance P in inducing inflammation and platelet activation. In agreement to our findings, Jone et al. defined the role of substance P in the activation of platelet function and thrombus formation. They showed that substance P can stimulate platelet activation and aggregation, and that platelets contain substance P immunoreactivity that is released upon activation. In addition, inhibition or deficiency of the neuropeptide NK1 receptor 1 (NK1) of this neuropeptide resulted in substance P-induced platelet aggregation and inhibition of platelet responses to collagen, indicating that NK1 agonists are released from activated platelets and act as paracrine agonists.

To the best of our knowledge, this is the first report on tachykinin neuropeptide, substance P in patients with thalassemia. The observed high levels add a new mechanism to the already well established causes of platelet activation and hypercoagulability in thalassemic patients.
5. Conclusion

- Substance P and sP-selectin might have a role in platelet activation and subsequent hypercoagulability in thalassemic patients.
- If proved by large scale studies, P-selectin and substance P could be novel targets in preventing the complications of thalassemia.

References