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ORIGINAL RESEARCH REPORT

Role of red blood cells "annexin V" and platelets "P-selectin" in patients with thalassemia

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Abstract

Objective/Background: Certain hemostatic anomalies found in patients with thalassemia suggest the existence of a chronic hypercoagulable state. Several etiologic factors may play a role in the pathogenesis of the hypercoagulable state in those patients. One of these factors is abnormal thalassemic red blood cells (RBCs), which may provide a procoagulant. To substantiate these findings, we measured the ability of RBCs from thalassemia patients to bind annexin V with increased fraction of platelets carrying the activation marker CD62P (P-selectin). To study the expression of RBC annexin V and platelets P-selectin in study patients (those with thalassemia major, thalassemia intermedia, thalassemia minor) and control group, four-color flow cytometry was performed and the correlation between these two markers was evaluated.

Methods: A case–control study was conducted on 50 β-thalassemia patients (10 patients with thalassemia minor, 30 patients with thalassemia major, and 10 patients with thalassemia intermedia, with 10 normal adult volunteers as a control) from June 2016 to March 2017. Flow cytometry was used to study the expression of anionic phospholipids (Annexin V) on the RBCs and CD62P (P-selectin) on the activated platelet.

Results: The mean expression of annexin V in patients with thalassemia major and intermedia was significantly higher than that in the control group and patients with thalassemia minor. Although the mean expression was higher in patients with thalassemia intermedia than in those with thalassemia major, it was not statistically significant.

Conclusion: The mean expression of platelets P-selectin in patients with thalassemia major and thalassemia intermedia was significantly higher than that in controls and patients with thalassemia minor. However, its expression was significantly higher in patients with thalassemia

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intermedia than in those with thalassemia major. Annexin V also showed a positive correlation with P-selectin, and both markers positively correlated with regularity of blood transfusion.

Introduction

Thrombophilia in patients with thalassemia is explained by many factors related to thromboembolic disorders such as endothelial cell stimulation, abnormal red blood cell (RBC) membrane, and platelets activation [1,2].

Phosphatidylserine may also play a role in directing vascular damage in thalassemia and both erythrocyte and platelets extracted from patients with β-thalassemia usually carry more reactive oxygen species and have lower glutathione levels, suggesting increased oxidative stress [3,4].

These changes induce two thrombophilic processes, thrombin generation and platelets activation, as phosphatidylserine expression is linked to platelets activation markers [5,6].

Many studies have demonstrated that patients with thalassemia show features of increased platelet aggregation and shortened platelets life span due to amplified platelet consumption, which in turn can be related to chronic thrombophilia or active thrombotic disorders [7].

Flow cytometry is used to study activation of chronic platelets in β-thalassemia major and thalassemia intermedia using CD62P (P-selectin) and CD63 as activation markers expressed in fraction of platelets [8], as well as with procoagulant activity of thalassemic RBC marked by annexin V [9].

The relation between annexin V and CD62 or CD63 may explain the interaction between platelets activation and the procoagulant surface membrane in thalassemia [9].

The aim of this research was to study the expression of RBC annexin V and platelets P-selectin in patients with thalassemia major, thalassemia intermedia, thalassemia minor, and in a control group using four-color flow cytometry and to evaluate the correlation between these two markers.

Materials and methods

Patients

A case—control study was conducted on 50 thalassemic patients (30 patients with thalassemia major, 10 with thalassemia intermedia, and 10 with thalassemia minor), alongside 10 age- and sex-matched controls. The aforementioned patients were attending Al Karama Teaching Hospital (Baghdad, Iraq) for receiving blood transfusion and treatment from June 2016 to March 2017. A full medical history review of each patient was performed, especially history of blood transfusion and physical examination, to exclude patients with serious complications. All patients were investigated within 6 h for the expression of RBCs (annexin) and activated platelets (P-selectin) using four-color flow cytometry (Partec Cyflow Cube 6; Sysmex Europe, Norderstedt, Germany).

Flow cytometric platelet analysis was performed on 100 μL of platelet-rich plasma pipetted into a tube, with gating of the cells of interest (RBC and activated platelets) performed depending on the forward/side scatter (FSC/SSC) gate. A marker expression is considered positive when the percentage of positive apoptotic RBCs is equal or greater than 20% while the activated platelet P-selectin expression is greater than 16.8% [10,11]. Oral informed consent was obtained from all patients. This study was approved by the Ethical Committee of Board Community (Institutional Review Board-College of Medicine, Al-Nahrain University) and conforms to Helsinki Declaration.

Statistical analyses

Statistical analyses were performed using SPSS (version 17.0 for Windows; SPSS Inc., Chicago, IL, USA). All p values <.05 were considered statistically significant.

Results

This study revealed that mean expression of RBCs annexin V and platelets P-selectin in thalassemia minor was comparable to their expression in normal control volunteers. By contrast, the mean expression of these in patients with thalassemia major and thalassemia intermedia was significantly higher than that in controls and patients with thalassemia minor. A comparison of the expression of annexin between patients with thalassemia major and thalassemia intermedia showed that it was higher in patients with thalassemia intermedia than in those with thalassemia major, but it did not reach statistical significance; however, P-selectin on platelets was significantly higher in patients with thalassemia intermedia than in patients with thalassemia major (Table 1).

In addition, this study showed that there was a significant positive correlation between annexin V and P-selectin (p < .005; Fig. 1). There was also a significant positive correlation between duration from the last transfusion (weeks) and annexin% (r = .411, p = .014) and P-selectin% (r = .461, p = .010), respectively.

Discussion

Our study results demonstrate that the expression of procoagulant annexin on the RBC membrane and platelets P-selectin is higher in patients with thalassemia major and intermedia compared to patients with thalassemia minor and controls. These results are comparable to other studies and can be explained by the fact that expression of annexin is closely related to excess production of the α-globin subunits, observed markedly in patients with thalassemia major and thalassemia intermedia. These inclusion bodies will

Table 1 The expression of annexin and P-selectin in studied groups.

Studied groups	Annexin V expression, %				P-selectin expression, %		
	N	Mean ± standard error	Median	Range	Mean ± standard error	Median	Range
Thalassemia minor	10	3.94 ± 0.948	3.99	0.64—9.11	5.25 ± 3.912	4.33	1.10—11.45
Thalassemia intermedia	10	85.45 ± 2.452	82.02	75.34—96.73	87.20 ± 8.091	87.50	70.98—95.86
Thalassemia major	30	78.56 ± 2.280	75.73	50.51—99.15	73.20 ± 12.548	73.52	41.82—94.72
Normal volunteers	10	3.90 ± 0.816	3.21	0.65—7.98	4.76 ± 0.823	4.31	0.76—9.80

Note. Thalassemia minor is significantly different from thalassemia intermedia ($p < .005$) and thalassemia major ($p < .005$); thalassemia intermedia is significantly different from thalassemia minor ($p < .005$) and control ($p < .005$); thalassemia major is significantly different from thalassemia minor ($p < .005$) and control ($p < .005$); control group is significantly different from thalassemia intermedia ($p < .005$) and thalassemia major ($p < .005$); p value for both Annexin and P-selectin < .005.

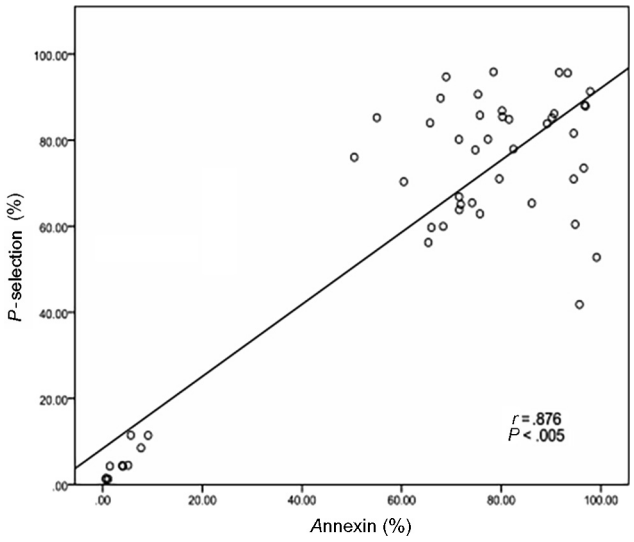

Fig. 1 Correlation between annexin V and P-selectin expression ($p < .005$).

undergo auto-oxidation with the production of hemichromes that stimulate heme disintegration and release toxic iron species and reactive oxygen radicals that will oxidize membrane proteins [12,13]. This eventually results in the overexpression and exposure of anionic phospholipids, such as phosphatidylethanolamine and phosphatidylserine [6,14], which will facilitate thrombin generation and expression of annexin. These changes will result in increased adhesion and aggregation of RBC, thus forming an RBC cluster that requires high shear stress for separation, thereby increasing the prothrombotic state in those patients [15].

Similarly, the expression of phosphatidylserine in thalassemia major and intermedia was highly associated with platelet activation markers through amplification of thrombin generation [6]. This chronic platelets activation state was confirmed by increased expression of P-selectin and CD63 [6], which may participate in platelet aggregation and shortened platelet survival owing to platelet consumption, and in turn may be associated with active thromboembolic disease or chronic hypercoagulable state [7,8,16].

We hypothesized that there is a causative relationship and an important association between RBC membrane anomaly manifested as an overexpression of annexin V and platelet overexpression of P-selectin, and that this correlation may result in vivo platelet activation and participate in the formation of hypercoagulability state. This was confirmed by Mannucci et al., who reported that the RBCs from patients with thalassemia major were an important stimulant for the activation of platelets in this patient group [18,19].

Similar results were also reported by other studies, which found a highly significant correlation between the number of RBC-bound annexin V molecules and the fraction of CD62P (P-selectin) or $CD63^{+}$ on activated platelets. These procoagulant proteins were simultaneously overexpressed in thalassemia major and thalassemia intermedia, which may explain the high incidence of thrombotic event in those patients [4,8,9]. Furthermore, similar to many studies, our study found that both annexin V and P-selectin overexpression was increased with increase in the age of patients with thalassemia, suggesting the increase in the defect of thalassemic RBCs that occurs over time [8,9].

Moreover, it was found that most pathological features of RBCs were reversed to normal range after blood transfusion, which explains the higher level of annexin in patients with thalassemia intermedia as they are not regularly transfused, leading to more abnormal cells being detected in their circulation and thus being more liable for thrombosis [19].

Conclusion

There was an overexpression of these procoagulant proteins that enhances the hypercoagulable state, resulting in accumulative abnormalities and close correlation of prothrombotic markers annexin V on RBC membrane and P-selectin on activated platelets in patients with thalassemia major and thalassemia intermedia but not in patients with thalassemia minor or controls. Moreover, patients with thalassemia intermedia showed higher expression of both markers. Therefore, it can be concluded that they are more liable for thromboembolic events.

Conflicts of interest

The authors have no conflicts of interest to declare.

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Further reading

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