Assessment of Circulating Red Cell and Platelet Microparticles Levels in Children with Non-transfusion Dependent Beta-Thalassemia

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Abstract

Objectives: To evaluate the circulating red cell and platelet microparticle percentages in children with Non-Transfusion-Dependent Thalassemia (NTDT) and its possible contribution to hypercoagulability.

Methods: Case-Control study, involved Fifty NTDT children from Alexandria University Children's Hospital and 50 age- and sex-matched healthy children were enrolled in this study. The children were recruited from the Hematology Oncology Unit at Alexandria University Children's Hospital, Alexandria, Egypt. The studied protocol obtained approval from the local ethical research committee of Alexandria University (Serial Protocol Number 0201580 on November 18, 2021).

Results: The percentage of Erythrocyte derived Micro-Particles (EMPs) and Platelet derived Micro-Particles (PMPs) were significantly higher in NTDT patients compared with healthy controls. Serum B-Type Natriuretic Peptide **(**NT-proBNP) was higher in NTDT patients compared to control; however, it didn't reach a statistical significance.

Conclusions: The study demonstrated significantly higher percentages of EMPs and PMPs in NTDT patients compared to the control group which may contribute to hypercoagulable state of the disease. Measurement of NT-proBNP can be used as a screening test for early detection of pulmonary hypertension in NTDT patients.

Keywords: Non-transfusion; Thalassemia; Microparticles.

Introduction

Beta-thalassemia syndromes are a group of heterogeneous autosomal recessive hereditary anemia resulting from defective synthesis of β-globin chains of adult hemoglobin A, leading to a range of phenotypes from severe anemia to clinically asymptomatic individuals. There is a wide variety of clinically distinct thalassemia syndromes;[1,2] the Thalassemia International Federation guidelines (2022)[3] put a new classification for thalassemia based on clinical severity and transfusion requirements into two main groups: transfusion-dependent and non-transfusion dependent.

A hypercoagulable status has been identified in thalassemia, especially in NTDT. Hypercoagulability in thalassemia is a multifactorial condition recognizing platelet abnormalities and pathologic red blood cells as crucial factors responsible for thrombotic events. Oxidative stress of thalassemic Red Blood Cells (RBCs) is associated with the expression of negatively charged phospholipids on their outer membrane leaflet, which can increase thrombin generation.[4,5] Under stress, apoptosis, or activation, cells release Extracellular Vesicles (EVs), submicrometric bioactive membrane vesicles. Extracellular vesicles are categorized by size into apoptotic bodies, Microparticles (MPs, which are large EVs), and exosomes (small EVs). Microparticles bud off from the plasma membrane, with 70-90% of circulating MPs being platelet-derived. Recently EVs have been explored as biomarkers for diagnosing clinical complications of various diseases.[6] In hematological disorders, they could be potential prognostic biomarkers for hypercoagulability especially in the context of thalassemia.[7]

Despite having acceptable hemoglobin levels and being transfusion-independent, patients with NTDT experience most thalassemia-related complications more frequently than those with TDT. These patients often develop thromboembolic events, such as deep vein thrombosis, portal vein thrombosis, pulmonary thromboembolism, and cerebral thrombosis, that are uncommon in TDT and typically manifest at an older age.[8] The prevalence of thrombotic events can reach up to 20% in NTDT patients compared with less than 1% in TDT patients.[9]

The risk of thrombosis is significantly increased by splenectomy in thalassemia patients.[10] Splenectomy is associated with decreased clearance of damaged RBC, chronic platelet activation, and increased MPs shedding.[11] Silent thrombosis can occur as subclinical thrombi in the brain and pulmonary vasculature.

Despite the increasingly recognized role of echocardiography as an initial screening tool to suspect Pulmonary Hypertension (PHT), cardiac catheterization is the gold standard technique.[12] However, supplementary data that are useful in the evaluation of PHT in thalassemia patients include markers of right heart dysfunction, such as an amino-terminal fragment of proB-type natriuretic peptide (NT-proBNP),[13] which is a hormone released in response to cardiomyocyte stretching. In the early phases of cardiac involvement, the level of NT-proBNP increases before an increase in diastolic pressure. Measurement of NT-proBNP provides diagnostic and mechanistic information concerning the development of PHT in thalassemia, and its level is a strong indicator of PHT in these patients.[14]

The present study aims to evaluate the circulating red cell and platelet microparticle percentages in children with Non-Transfusion-Dependent Thalassemia (NTDT) and its possible contribution to hypercoagulability.

Methods

This study was carried out on 50 β-NTDT children selected from regular attendants of the Pediatric Hematology Outpatient Clinic of a tertiary healthcare facility affiliated with a University Hospital, as well as 50 sex- and age-matched healthy children from November 2022 till May 2023. Patients' ages ranged between 8 and 16 years, with a mean of 10.42 ± 2.86 years. The diagnosis of NTDT was based on clinical symptoms and signs (delayed age of presentation > 2years, mild to moderate anemia, mild hepatomegaly and splenomegaly); frequency of blood transfusions (fewer than six red blood cell units over the last six months); and complete blood picture and diagnostic investigations of beta-thalassemia including hemoglobin electrophoresis and, if available, PCR β-globin gene analysis from patients' files.[15] Any patient with a history of blood transfusion in the past 3 months, positive for hepatitis C virus infection, or having a family history of thrombotic or bleeding disorders was excluded from the present study.

Ethical approval: After approval of the Ethics Committee at the Faculty of Medicine, Alexandria University, Egypt, IRB No. 0001298, FWA No. 00018699. EC Serial Protocol Number 0201580 on November 18, 2021, a written informed consent to participate was obtained from every participant sharing in the study (parent or surrogate in case of patients under 18 years old).

History and Clinical examination: All studied patients were subjected to a detailed medical history and full clinical examination, with special emphasis on chest and cardiac examination. An abdominal examination to assess hepatic size and splenic status; a lower limb examination is performed for evidence of thrombosis.

***Routine Laboratory investigations*:** include Complete blood counts, serum chemistry, serum ferritin level (on Centaur XPT Siemens automated analyzer), and D-dimer level (on Sysmex Siemens automated analyzer). Hypercoagulation is considered when D-dimer is more than or equal to 550 ug/L).[16] Platelet aggregation test

was performed by aggregometer with agonists adenine 5-diphosphate (ADP) in a ratio of 1:10 volume of the agonist. Using monoclonal antibodies, serum B-type natriuretic peptide (NT-proBNP) levels were quantitatively determined using an enzyme-linked immunosorbent assay (ELISA).

Circulating red cell and platelet microparticles preparation and analysis:[17] *Samples for microparticle detection* were collected in an atraumatic technique using larger needles to avoid sheer stress and endothelial activation. Two milliliters of venous blood from each patient were withdrawn on 3.2% sodium citrate in vacutainer tubes. Samples were processed within 15 min of collection.

***Isolation of microparticles*:** Microparticles (MP) were isolated by centrifugation of whole blood at 1,500×g for 15 min at RT. The Platelet poor plasma (PPP) was separated in another tube while the residual amount of plasma above the cell pellet was discarded. The PPP was then centrifuged at 14,000×g for 2 min at 20 °C to obtain platelet free-plasma (PFP). Afterward, a microparticle pellet was obtained from the PFP by re-centrifugation at 14,000×g for another 45 min at 4 °C. Subsequently, the supernatant was discarded, and the microparticle pellet was resuspended in PBS and vortexed.

Evaluation of MPs using flow cytometry: MPs were enumerated by high-sensitivity flow cytometry following standardization, Briefly, 30 µl PFP was incubated with the appropriate amount of specific monoclonal antibodies (mAbs) plus 10 µl of Annexin V. Monoclonal Ab included Ab to glycophorin A (GPA)(CD235a-FITC, for identification of red blood cell derived MPs), glycoprotein (GP) IIb/IIIa (CD41a-PE, for identification of Platelet-derived MPs). All monoclonal antibodies were purchased from BD biosciences, CA, USA.

Megamix-Plus FSC beads (BioCytex) beads were used , MPs identified as being between 0.3 and 1 µm beads. Platelet- (PMPs) and erythrocyte- (Ery-MPs), were defined as $AnnV^{+}/CD41^{+}$, $AnnV^{+}/CD235a^{+}$, respectively^{+}). After Ab labeling, the samples were acquired on BD FACSCanto II flow cytometer and the collected data were analyzed using FSCSDiva software (BD biosciences, CA, USA).

Flow cytometric analysis of MPs is presented in Figures 1.

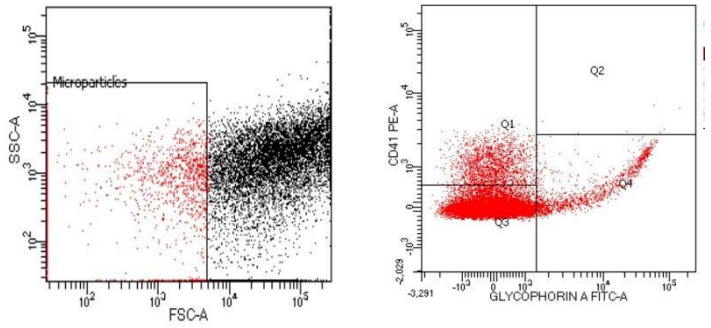

Figure 1: Flowcytometry analysis of erythroid derived microparticles (CD235a positive) and platelet derived microparticles (CD 41positive).

Statistical Methodology: Data analysis was carried out using the SPSS (Statistical Package for Social Science) program for statistical analysis (ver 25). Shapiro–Wilk test of normality was used to assess the distribution of the quantitative variables. The normally distributed data was analyzed using parametric statistics (mean ± SD, t-test), while the not normally distributed data used a non-parametric test (median, Mann-Whitney U test).[18] A nomogram is a concise graphical illustration of a prediction model that provides a numerical probability of a clinical event. This study uses a structured nomogram model based on the three key independent predictors (PMPs, EMPs, and Ferritin) to generate a prediction nomogram for activated coagulation. The nomogram was designed using the Orange Data Mining Toolbox software.[19] During sample size calculation, beta error was accepted up to 20%, with a power of study of 80%. An alpha level was set to 5% with a significance level of 95%. Statistical significance was tested at p-value <.05.[20]

Results

The study included 50 NTDT patients, 32 males, and 18 females; their ages ranged from 8 to 16 years, with a mean of 10.42 ± 2.86 years. Fifty age ($p = 0.701$) and sex-matched ($p = 0.309$) apparently healthy children were included as a reference group. Six (12%) of the patients were splenectomized. None of the patients had clinical evidence of pulmonary hypertension or thrombosis.

The demographic and laboratory data of NTDT patients and control group is presented in Table 1. No significant difference was noted between patients and control regarding age and sex. There was a statistically significant difference in hematological parameters including; Hemoglobin (Hb) level, RBCs count and reticulocytic counts between NTDT patients and control ($p < 0.01$). No significant difference in platelets count ($p = 0.325$), however nine patients (18%) had thrombocytosis (platelets count >450x10^6/µL). Serum ferritin level was significantly higher in NTDT patients ($p < 0.001$). Erythrocyte-derived MPs (EMPs) and platelet-derived MP (PMPs) percentages were significantly higher in NTDT patients ($p < 0.01$). NT-proBNP was higher in NTDT patients compared to control; however, it didn't reach a statistical significance.

Table 1: Comparison of Non-Transfusion Dependent Thalassemia patients and Healthy Reference Group.

	Group		**Test of**
Variables	**NTDT patients**	**Health Reference**	**significance**
	(n=50)	(n=50)	***p*-value**
Age (years)			
- Min-Max	8.00-16	8.00-16.00	$t_{(W)(df=87.850)}$=0.385
- Mean ± S.D.	10.42±2.86	10.61±2.01	*p*=.701 NS
Sex			
- Male	32 (64.00%)	27 (54.00%)	$\chi^2_{(df=1)}$=1.033
- Female	18 (36.00%)	23 (46.00%)	*p*=.309 NS
Hemoglobin (g/dL)			
- Min-Max	5.20-11.90	10.30-12.60	$t_{(W)(df=70.315)}$=20.635
- Mean ± S.D.	7.51±1.21	11.42±0.58	*p*<.001*
Red Blood Cell count (×10^6/µL)			
- Min-Max	2.36-5.70	4.50-5.44	$t_{(W)(df=58.661)}$=11.208
- Mean ± S.D.	3.78±0.68	4.92±0.21	*p*<.001*
Reticulocytes (%)			
- Min-Max	0.50-6.80	0.10-2.00	$t_{(W)(df=67.2001)}$=13.723
- Mean ± S.D.	3.72±1.41	0.72±0.55	*p*<.001*
Platelets count (×10^6/µL)			
- Min-Max	146.00-1153.00	190.00-437.00	$t_{(W)(df=62.585)}$=0.992
- Mean ± S.D.	330.20±207.68	299.06±78.09	*p*=.325 NS
Serum Ferritin (µg/L)			
- Min-Max	17.90-2086.00	20.00-62.80	$t_{(W)(df=49.076)}$=6.621

- Mean ± S.D.	441.41±433.89	35.00±12.07	p<.001*
Erythrocyte-derived Micro-Particles (%)			
- Min-Max	0.30-20.00	0.80-3.30	$t_{(W)(df=52.428)}$=3.898,
- Mean ± S.D.	4.52±4.67	1.90±0.78	p<.001*
Platelets-derived Micro-Particles (%)			
- Min-Max	1.00-28.10	0.90-3.30	$t_{(W)(df=51.652)}$=6.281
- Mean ± S.D.	6.09±4.43	2.11±0.73	p<.001*
N-Terminal Pro-B-Type Natriuretic Peptide (pg/ml)			
- Min-Max			$t_{(W)(df=98)}$=1.764
- Mean ± S.D.	98.48±17.51	91.82±20.15	p=.081 NS

n: Number of patients S.D.: Standard Deviation W: Welch's t-test

$\chi^{2:}$ Pearson Chi-Square df= degree of freedom *Statistically significant (p<.05)

NS: Statistically significant (p≥.05)

A statistically significant positive correlation existed between PMPs and platelet count (r=0.471, p=0.01). No significant correlation was encountered between EMPs and hematological and laboratory parameters (serum ferritin, and D dimer levels) (Table 2).

Table 2: Correlation between Erythrocyte-derived Micro-Particles, Platelets-derived Micro-Particles, and N-Terminal Pro-B-Type Natriuretic Peptide levels with different parameters in NTDT patients(n=50).

Variables	**r**	***p*-value**
Erythrocyte-derived Micro-Particles (%) vs.		
● Hemoglobin Level (g/dl)	0.140	.333
● Red blood cell count (x10^6/Ul)	0.202	.160
● Reticulocytes (%)	-0.228	.111
● Lactate Dehydrogenase (U/L)	0.134	.353
● Ferritin (ng/ml)	0.067	.645
● D-dimer (µg/L)	-0.024	.866
Platelets-derived Micro-Particles (%) vs.		
● Platelets count (×10^3/µL)	0.471	.001*
● D-dimer (µg/L)	0.121	0.402
● Ferritin (ng/ml)	0.226	.114
NT-proBNP level vs.		
● Hemoglobin Level (g/dl)	0.006	1.000

• Platelets count ($\times 10^3/\mu L$)	0.080	.582
• D-dimer level (µg/L)	0.137	.344
• Ferritin level (µg/ml)	0.051	.724
• EMPs percentage	0.098	.499
• PMPs percentage	0.028	0.845

r: Pearson's correlation coefficient

Platelet aggregation was tested using an ADP agonist and was normal in all patients, ranging between 75-85% with a mean of 80.20 ±3.61%. The mean D-Dimer level of patients was 490.4 ± 150 µg/L, ranging from 70 to 750 µg/L. 21 patients with levels >550 µg/L.

No statistically significant difference existed between patients with normal D-Dimer and those with high D dimer in hematological parameters, namely, Hb level (p=0.608), RBCs count (p=.305), White blood cell count (p=.090), Reticulocytes (p=.146), and platelets count (p=.882). Also, number of patients with thrombocytosis is not statistically significantly different between the two subgroups (p=.146) (Table 3).

No statistically significant differences were found between patients with normal level D-Dimer and those with high D-dimer as regards PMPs percentage (p=0.360) and EMPs percentage (p=.512). Serum ferritin and NT-ProBNP levels were statistically significantly higher in patients with high D-Dimer (p=, .012, 047; respectively) (Table 3).

Table 3: Hematological investigations, Microparticles, serum ferritin, and NTproBNP in the NTDT patients (n=50) compared based on D-Dimer level (hypercoagulable status).

Variables	**D-Dimer level**		**Test of significance**
	Normal D-dimer (<550 µg/L) (n=29)	**High D-dimer (≥ 550 µg/L) (n=21)**	***p*-value**
Hemoglobin (g/dL)			
- Min. – Max.	5.70 – 9.30	5.20 – 11.90	$t_{(df=48)}$=0.517
- Mean ± S.D.	7.59 ± 1.04	7.41 ± 1.43	p=.608 NS
Red Blood Cell count ($\times 10^6/\mu L$)			
- Min-Max	2.36 – 4.90	2.75 – 5.70	$t_{(df=48)}$=1.037
- Mean ± S.D.	3.70 ± 0.63	3.90 ± 0.74	p=.305 NS
Reticulocytes (%)			
- Min-Max	1.50 – 6.80	0.50 – 6.80	$t_{(df=48)}$=1.478
Mean ± S.D.	3.47 ± 1.29	4.06 ± 1.52	p=.146 NS
White Blood Cell Count ($x10^9$/L)			

- Min-Max	2.81 – 18.00	4.56 – 31.50	$t_{(df=48)}$=1.729
- Mean ± S.D.	8.01 ± 3.11	10.59 ± 7.19	*p*=.090 NS
Platelets count (×10^6/µL)			
- Min-Max	153.00 – 807.00	146.00 – 1153.00	$t_{(df=48)}$=0.149
- Mean ± S.D.	333.97 ± 154.63	325.00 ± 268.60	*p*=.882 NS
Thrombocytosis	5 (17.24%)	4 (19.05%)	Z=0.164
			p=.873 NS
Lactate Dehydrogenase (U/L)			
- Min-Max	164.00-410.00	190.00 – 420.00	$t_{(df=48)}$=2.251
- Mean ± S.D.	241.52 ± 68.72	287.81 ± 75.83	*p*=.032*
Platelets-derived Micro-Particles (PMP) (%)			
- Min. – Max.	1.00 – 14.00	2.00 – 28.10	$t_{(df=48)}$=0.925
- Mean ± S.D.	5.60 ± 3.22	6.77 ± 5.71	*p*=.360 NS
Erythrocyte-derived Micro-Particles (%)			
- Min-Max	0.30 – 20.00	0.50 – 20.00	$t_{(df=48)}$=0.660
- Mean ± S.D.	4.14 ± 4.13	5.03 ± 5.38	*p*=.512 NS
Serum Ferritin (µg/L)			
- Min-Max	17.90 – 1700.00	56.00 – 2086.00	$t_{(df=48)}$=2.604
- Mean ± S.D.	312.81 ± 336.22	619.00 ± 495.86	***p*=.012***
N-Terminal Pro-B-Type Natriuretic Peptide (pg/ml)			
- Min-Max	64.53 – 123.23	75.31 – 141.97	$t_{(df=48)}$=2.035
- Mean ± S.D.	94.32 ± 15.39	104.22 ± 18.97	***p*=.047***

n: Number of patients *S.D.: Standard Deviation* *t: Student's t-test*

Z: Z test for comparison of two independent proportions *df= degree of freedom*

**Statistically significant (p<.05)* *NS: Statistically significant (p≥.05)*

In the present study, serum ferritin level proved to be a statistically significantly "Good" discriminator of hypercoagulability (D-Dimer ≥ 550 µg/L) with Area under the ROC curve (AUC) = 0.740 (95% CI 0.596-0.853) (Z=3.305, *p*=.001). The diagnostic criterion using Youden index is the level of >277.3 µg/L with a Sensitivity of 80.95% (95% CI: 58.09% to 94.55%), Specificity of 68.97% (95% CI: 49.17% to 84.72%), Positive Predictive Value (PPV) of 65.38% (95% CI: 51.38% to 77.15%), Negative Predictive Value of (NPV) of 83.33% (95% CI: 66.70% to 92.58%), and statistically significant Overall accuracy of 74.00% (95% CI: 59.66% to 85.37%) (*p*=.0142). The risk (Odd's ratio) of being activated coagulation (D-Dimer ≥ 550 µg/L) when serum ferritin is >277.3 µg/L is 9.444 folds (95% CI 2.464-36.198).

A predictive model for hypercoagulability (D-dimer ≥ 550 µg/L) was built, including PMPs, EMPs, and Ferritin as independent predictors. These parameters were incorporated into the nomogram to predict activated coagulation. The value on each axis corresponding to a predictor matches a score on the top axis (points) when a

straight line (blue arrow) is drawn. After the summation of all points, a vertical line (red arrow) will be drawn from the total points axis to reach the corresponding linear probability of Activated coagulation. A screenshot of the predictive nomogram is presented in Figure 2.

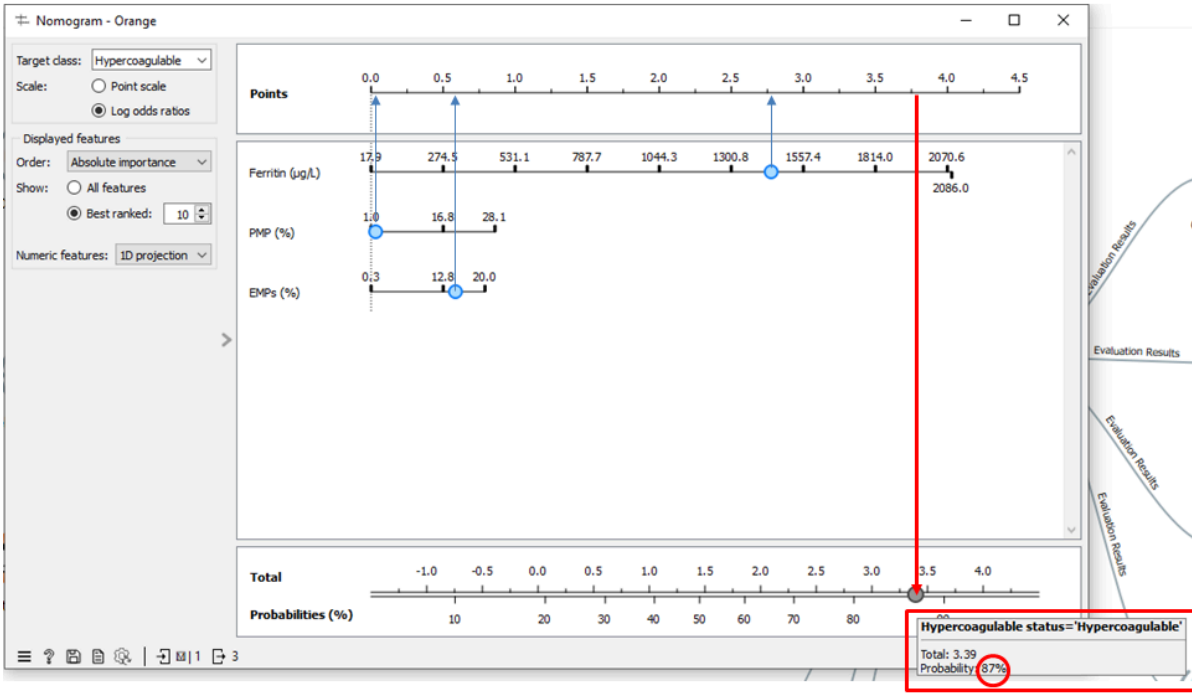

Figure (2): A prediction nomogram for hypercoagulability among NTDT patients.

A screenshot for one of the included patients with Activated coagulation with baseline serum ferritin (1442.6 µg/L), PMP (2%), EMP (14.9%), resulted in a prediction of activated coagulation in a probability of 87%.

Composite Area under the Receive Operator Characteristics (ROC) Curve using the Naïve Bayes statistical method was 71.1%, with a classification accuracy of 68.0% and precision of 63.2%. In a trial to test the accuracy of the model, we used the Monte Carlo data simulation model and generated a sample of 250 patients (based on the study data collected); this resulted in improvement in the Area under the ROC to 100%, classification accuracy to 98.4%, and precision to 98.4%. This finding may be a clue that this predictive model will be beneficial in predicting Activated coagulation if applied to a larger sample (Suppl. Figure 3).

Discussion

Patients with B thalassemia are at increased risk for hypercoagulability and thrombosis, significantly impacting morbidity and mortality. This phenomenon involves interactions between damaged blood cells, activated leukocytes and platelets, adhesive endothelial cells and coagulation factor dysregulation.[11,21] Pathological red blood cells and chronic platelet activation are the key factors causing hypercoagulability.[11,21]

Circulating cell-derived vesicles have been reported in thalassemic patients, especially splenectomized ones; these include apoptotic bodies. Various studies have examined the profile and quantity of circulating MPs focusing on their cellar origin and procoagulant properties and revealed a notable presence of plasma MPs primarily released from platelets and damaged red cells which express negatively charged phospholipids.[7,22,23] In patients with thalassemia, various studies revealed a notable presence of plasma MPs primarily released from platelets and damaged RBCs which express negatively charged PS. These MPs were found to trigger platelet activation and aggregation, contributing to thrombi formation.[7]

The present study evaluated EMPs and PMPs in 50 NTDT patients and 50 age and sex-matched normal children. EMPs were significantly higher in the thalassemic group compared to the control group. This may

contribute to initiation of hypercoagulability with other pro-coagulant factors in these patients. Several investigators[4,5,24-26] agreed with this conclusion.

We could not elicit a correlation between EMPs level and some studied parameters, including Hb level, RBCs count, hemolytic markers (reticulocytic count and LDH), and serum ferritin. However Shahin et al.[24] in their study on pediatric Thalassemia Intermedia (TI) patients, reported a significant positive correlation between EMPs and ferritin levels and a significant negative correlation between EMPs and Hb levels. However, no correlation was encountered between EMPs and RBC count or LDH level.

Mowafy et al. (2016)[25] and Youssry et al., (2017)[4] reported a negative correlation between the Level of EMPs and Hb level only. The variability in correlation results can be explained by differences in the studied patients' number, age, and/or clinical characteristics.

Platelet microparticles are procoagulant subcellular vesicles released from activated platelets and facilitate coagulation.[27] Increase in platelet-derived EVs has been identified as predictive biomarkers for thromboembolic events in both patients with Thalassemia Major (TM) and TI.[4,23]

In the present study, the mean PMP level was significantly higher among NTDT group than the control group. This is consistent with other studies.[4,5,9,26,28,29] In the present study, there was a significant positive correlation between PMPs and platelet count, suggesting chronic platelet activation and release of microparticles. Abdelaziz et al. (2022)[29] reported no statistically significant correlation between PMPs and platelet count in their research; however, they reported a positive correlation between PMPs level and platelet count in splenectomised patients.

Enhanced platelet aggregation and increased platelet activation markers indicate platelet activation in the body.[30] In the present study, platelet aggregation showed a normal range in all patients (75-85% with a mean of 80.20±3.61%.) None had hyperaggregation (more than 90 %).[23] Similarly, Zahedpanah et al. (2018),[31] reported normal platelet aggregation by ADP agonist (80.4±9.4) in 36 β-TI young adult patients. They explained this by chronic platelet activation in β-TI, which might prevent the agonists' further stimulation of the platelets; actually, the activated platelets become refractory to additional stimulation. Results of platelet aggregation vary in different studies and even among patients. An increased,[22,23] decreased[32] or even normal reactivity was reported.[10,33] Variation in results in different studies can be attributed to differences in laboratory techniques, disease characteristics, or racial differences.[33]

The high plasma levels of D-dimer can be taken as an indirect index of thrombin activity. D-dimer is a marker for fibrin formation and fibrinolysis, hence thrombotic risk in these patients.[29] In the present study, the mean values of D-dimer in patients were near normal (less than 550 µg/L); however, 21 patients had higher levels above 550 µg/L, and 24% of them were splenectomized.

None of the included patients had thrombotic manifestations. This can be explained by the fact that the age of occurrence of thrombosis is usually 18 years or above. In the most extensive clinical study that analyzed data from 8,860 thalassemia patients (6,670 TM and 2,190 TI), it was reported that age beyond 20 years and splenectomy are the main risk factors for developing thrombosis in their study group.[34]

Silent thrombosis can occur as subclinical thrombi in the microvasculature of the lungs and brain. Pulmonary hypertension is well documented in NTDT and carries a poor prognosis in older age that has a high probability of occurrence.[35]

Echocardiography remains the cornerstone screening test for the diagnosis of pulmonary hypertension.[36]

Serum level of NT-proBNP has been proposed as a non-invasive biomarker for early detection of pulmonary hypertension.[37]

Pro-type natriuretic peptide (NT-proBNP) is a hormone released from cardiomyocytes. The main stimulus for the increased synthesis and secretion of NT -proBNP is myocardial wall strain.[38]

The hormone has acquired significance in several cardiovascular disorders, and its level correlates with the severity of pulmonary pressure elevation.[14]

In the early phases of cardiac involvement, its level increases before an increase in diastolic pressure and right ventricular dysfunction. NT-proBNP can be used as a screening test to detect PHT.[38] We assessed proBNP levels in fifty NTDT and fifty controls. The mean level was higher in patients compared with control, although

the difference is statistically not significant. This is consistent with Safniyat et al;[39] other workers reported significantly higher NT-proBNP levels.[40,41] However, these studies were in adult thalassemic patients with TM and TI. In an earlier study, Voskaridou et al (2007)[14] reported significantly elevated NT-ProBNP levels in patients with documented PH hypertension. However, even patients without PH had high level compared with controls. They concluded NT-ProBNP is a strong indicator of PH.

Although we didn't elicit a correlation between NT-proBNP and D-dimer levels in the studied patients, it is worth mentioning that patients with elevated D-dimer levels had significantly higher NT-proBNP levels than those with normal D-dimer. D-dimer is a marker of fibrin formation and hence thrombotic risk, suggesting the occurrence of microthrombi in pulmonary circulation leading to right ventricular strain and increased synthesis and secretion of NT-proBNP in those patients.

In the present study, Serum Ferritin level proved to be a good discriminator of hypercoagulability with a sensitivity of 80.95% (95% CI: 58.09% to 94.55%), Specificity of 68.97% (95% CI: 49.17% to 84.72%), Positive Predictive Value (PPV) of 65.38% (95% CI: 51.38% to 77.15%), Negative Predictive Value of (NPV) of 83.33% (95% CI: 66.70% to 92.58%), and statistically significant Overall accuracy of 74.00% (95% CI: 59.66% to 85.37%) (p=.0142). Youssry et al. (2017)[(4)] reported a significantly higher ferritin levels in patients with thromboembolic events. In an analysis based on data from 584 TI patients enrolled in OPTIMAL CARE study showed that serum ferritin ≥1000 ng/L, age>20 significantly increased the risk of thrombosis.[34]

No statistically significant difference was found between patients with normal and those with high D-Dimer (hypercoagulable) as regards hematologic variables or microparticles percentages (Table 3), which may be attributed to small sample size. A prediction nomogram with high performance based on the three key predictors of hypercoagulability (Ferritin, EMPs, PMPs) was structured and introduced as simple, on invasive and rapid tool for anticipating hypercoagulable state in NTDT and proved to be reliable for early prediction of hypercoagulability in TI patients.

The present study has potential limitations including the relatively small sample size, and being done at a single-center.

Conclusion

No evident thrombotic event was detected in the present study, so, no definite management plan could be suggested. Measurement of NT-proBNP can be used as a screening test for early detection of pulmonary hypertension.

Recommendations

Assessment of EMPs and PMPs might provide a means for early detection of hypercoagulability and thrombotic risk. Regular cardiac evaluation, including adequate NT-proBNP assessment, is recommended tool for early detection of pulmonary hypertension particularly in high-risk patients. Further large sample size study, with event rate of thrombo-embolic manifestation could be the mile-stone for these treatment strategies.

Disclosure

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