Platelet derived micro particles and the risk of pulmonary hypertension in Egyptian patients with thalassemia major

Mariana Rady Moawad [1], Hanaa Hassan Eldash[1], Sherin Khamis Hussein[1], Mohammed Mansour Abbas Eid[2]

[1] Department of Pediatrics, [2] Department of of Clinical Pathology, Faculty of Medicine, Fayoum University, P.O. Box 63514, Fayoum, Egypt
mma21@fayoum.edu.eg

Abstract: Chronic platelet activation usually accompanies this hypercoagulable state noticed in thalassemia. The associated thrombotic risk is partially attributed to the presence of high levels of membrane-derived microparticles (MPs) originating from activated platelets. The level of circulating platelet-derived microparticles (PDMPs) as a risk factor for pulmonary hypertension in Egyptian children with β-thalassemia major is a subject of interest. Forty β-thalassemic children and thirty age- and sex-matched healthy subjects were enrolled in this study. CBC, serum ferritin and serum level of PDMPs were measured. Assessment of systolic ventricular function and pulmonary artery pressure was done using Doppler Echocardiographic study. Serum level of PDMPs was significantly elevated in thalassemic patients compared to healthy controls. Fifty percent of our cases had mild to moderate pulmonary hypertension. Splenectomized thalassemics had higher level of thrombocytosis and higher mean of pulmonary pressure compared with non-splenectomized counterparts. PDMP was higher in patients with pulmonary hypertension with significant difference ($p<0.05$). Platelet-derived microparticles (PDMPs) may be implicated in vascular dysfunction and the risk of pulmonary hypertension in thalassemia patients. Their quantification could provide utility for early detection of cardiovascular abnormalities.

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1. Introduction

One of the inherited disorders of hemoglobin synthesis is β-thalassemia which is characterized by ineffective erythropoiesis, different grades of defective β-chain manufacture, an inequality in α/β-globin chain synthesis and anemia (Cappellini et al., 2012). Though the life expectation of β-thalassemia patients has significantly improved over the last few years, they still suffer from several complications of this inherited disease (Taher et al., 2008). Owing to hemostatic abnormalities, a hypercoagulable state exists in thalassaemic patients in early childhood, which might contribute to the cardiac and pulmonary abnormalities and the thrombotic events which happen far along (Eldor et al., 2001).

Chronic platelet activation usually accompanies this hypercoagulable state that has been noticed in thalassemia. The associated thrombotic risk is partially attributed to the presence of high levels of membrane-derived microparticles (MPs), stemming from activated platelets (Tantawy et al., 2013). Platelet-derived microparticles are coagulant subcellular vesicles released from activated platelet (previously called platelet dust). Platelet factor 3 is mentioned as the ability of both platelet and PMPs to facilitate coagulation (Nieuwland et al., 2002).

Microparticles (MPs) become more procoagulant and more prominent when β-thalassemia patients are intensively transfused, and this may contribute to increased risk of pulmonary hypertension, vascular dysfunction and aortic wall stiffness detected in thalassemia patients. So, their quantification may provide efficacy for early recognition of cardiovascular abnormality (Agouti et al., 2015).

The aim of this study was to assess the level of circulating platelet-derived microparticles (PDMPs) as a risk factor for pulmonary hypertension and hypercoagulable status in Egyptian children with β-thalassemia major.

2. Patients and Methods

This case-control study included 40 patients diagnosed as β-thalassemia major (21 males and 19 females with a male to female ratio 1.1:1), 13 patients were splenectomized and 27 patients were non-splenectomized. Patients were recruited from regular attendants of the Pediatric Hematology Clinic, Fayoum University Hospital during the period from June to December 2016. Thirty age- and sex- matched healthy subjects were enrolled as a control group. An informed consent was obtained from the guardian of each patient and control before participation. This

study was reviewed and approved by Fayoum Faculty of Medicine Research Ethical Committee.

All patients in the study were subjected to complete thorough history taking and physical examination. Laboratory investigations were done in the form of complete blood picture (Sysmex KX-21N Automated Hematology Analyzer, Sysmex Europe GmbH, Hamburg, Germany), serum ferritin level (ARCHITECT i1000SR immunoassay analyzer, Abbott, Illinois, U.S.A.) and measurement of serum platelet-derived microparticles using double-sandwich ELISA technique, using commercially available kit (My Bio Source, San Diego, USA). ELISA plate absorbance was read by 800 TS absorbance reader (BioTek Instruments, Inc., Winooski, VT, USA).

Also, Echocardiographic (DC-70, Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Nanshan, China) study was done for cases in the form of assessment of: estimated systolic pulmonary artery pressure, ejection fraction, fraction shortening and tricuspid regurgitant jet velocity.

Reference ranges of echocardiographic parameters for Egyptian healthy children:

- EF (Ejection Fraction): 55% -84%
- FS (Fraction of shortening): 25 -55%
- ESPAP (Estimated systolic pulmonary artery pressure): 15 - 25 mmHg, where pulmonary hypertension is present if ESPAP>25 mmHg at rest, sub classified into:

- Mild (25 - 40 mmHg)
- Moderate (41 - 55 mmHg)
- Severe (> 55 mmHg) (Park, 2014)

3. Statistical Analysis

Data were collected and coded to facilitate data manipulation and double entered into Microsoft Access and data analysis was performed using SPSS software version 18 in windows 7. For quantitative parametric data; in-depended student t-Test was used to compare measures of two independent groups of quantitative data. One-way ANOVA test was used in comparing more than two independent groups of quantitative data. Kruskal wallis test was used in comparing more than two independent nonparametric groups. Mann-whitney test was used in comparing two independent nonparametric groups. For qualitative data; Chi square test was used to compare two of more than two qualitative groups. Bivariate pearson correlation test was used to test association between variables. The level $P \leq 0.05$ was considered the cut-off value for significance.

4. Results

4.1 Basic Demographic and clinical data of study groups

This case-control study included 40 patients as the case group, recruited from attendants of the pediatric hematology clinic and 30 age- and sex-matched healthy subjects as a control group. The mean age of the study group was 7.9 years with a range between 6 months and 13 years. Females constituted 52.5% of the group while males constituted 47.5%. The majority of the cases were from rural areas 75%, also most of the cases had positive consanguinity (72.5%) and had similar conditions in their families (70%). In this study 30% of the cases had been splenectomized, 12.5% had history of infection with viral hepatitis. None of the cases had any apparent thromboembolic events or vascular disease.

Almost half of the cases (45%) were using Deferasirox, 20% were using Deferoxamine, 15% were using Deferiprone, 2.5% of cases were using combined therapy: Deferiprone and Deferasirox. And 17.5% of the cases does not use any iron chelating agent. There was no relation between serum level of PDMPs and the use of different types of iron chelating agents. Cases had significantly lower hemoglobin and higher both total leucocytic count (TLC) and serum PDMPs compared to controls (Table 1).

Table (1): Comparisons of laboratory findings among cases and controls

Parameter	Cases (n=40)		Controls (n=30)		p
	Mean	SD	Mean	SD	
Hb level	**6.2**	1.2	**10.6**	1.2	**<0.001**
Platelet count	338.8	149.3	346	87.8	0.8
Total leucocytes count	**12.7**	8.5	**8.8**	2.9	**0.02**
Serum Platelet-derived micro-particles level	**1.85**	2.04	**0.71**	0.51	**<0.001**

4.2 Relation between PDMPs and serum ferritin level

Among controls, 24 children (almost the half) were found to have mild anemia (48%). The anemic controls appeared to have iron deficiency anemia; reduced both serum iron<50mcg/dl and ferritin<10ng/ml with elevated TIBC>350mcg/dl.

Serum ferritin was < 1000ng/dl in 25% our thalassemic cases and 1000-2500 ng/dl in 35%, while 40% of the cases had high levels > 2500 ng/dl. There

was a statistically significant relation between serum level of PDMPs and serum ferritin; with a higher mean value among thalassemic cases with serum ferritin > 2500 ng/dl (p-value 0.02) (Table 2). These data showed that increase in serum PDMPs is associated with concomitant increase in serum ferritin.

Table (2): Relation between PDMPs and ferritin serum level

Ferritin	Serum level of PDMPs		p
	Mean	SD	
< 1000 ng/dl	0.65	0.7	0.02
1000 -2500 ng/dl	1.5	1.4	
>2500 ng/dl	1.8	1.7	

4.3 Correlation between serum level of PDMPs and selected laboratory investigations

There was no statistically significant correlation between serum Platelet-derived micro-particles level and disease duration, age of onset and frequency of blood transfusion. Also there was no statistically significant correlation between serum Platelet-derived micro-particles level and other laboratory investigations including: (HB, PLT and TLC) (Table 3). Splenectomized patients are known to have increased serum levels of PDMPs. So next, we studied PDMPs together with some selected lab parameters in splenectomized patients compared to non-splenectomized ones. As shown in Table 4, we found that splenectomized patients had significantly higher platelet count, serum PDMPs and ESPAP than non splenectomized patients ($p<0.05$).

Table (3): Correlation between serum level of PDMPs and other investigations

Parameter	Serum Platelet-derived micro-particles level	
	r	p-value
Hb level	-0.13	0.4
Platelet count	0.21	0.2
Total leucocytes count	0.03	0.9
ESPAP	0.21	0.2

Table (4): Different Investigations in relation to splenectomy

Investigations	Splenectomy				p-value
	No (n=28)		Yes (n=12)		
	Mean	SD	Mean	SD	
Hb level (g/dL)	6.2	1.3	5.9	1	0.6
Platelet count (cells/mm^3)	297.4	74.3	435.5	225.6	0.006
TLC (cells/mm^3)	11.2	6.7	16.4	11.3	0.08
Serum PDMPs	0.73	0.52	2.37	2.22	0.029
Serum ferritin level	1867.9	1257.4	2485.5	1236.4	0.128
ESPAP*	24.8	5.9	33.8	10.3	0.002

* Estimated systolic pulmonary artery pressure

Table (5): Description of Echo parameters among thalassemia cases

Variable	Minimum	Maximum	Mean	SD
EF (%)	60	83	68.3	6.2
FS (%)	25	51	37.8	5.6
ESPAP (mmHg)	18	50	27.1	8.3
TR	0	40	17.2	8.8

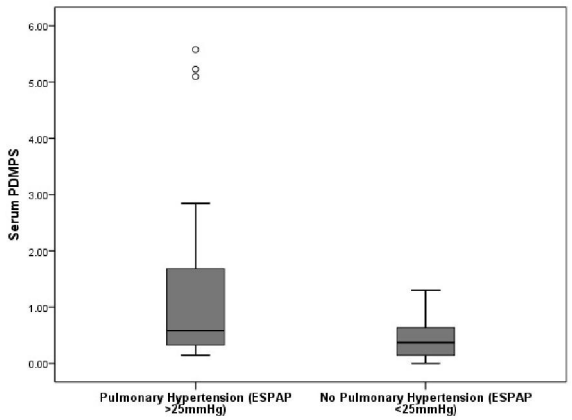

Figure (1): Serum levels of PDMPs in relation to pulmonary artery pressure. Mean serum PDMPs in Pulmonary hypertension (ESPAP >25) (N=19) is 1.51 ± 1.82, while in No pulmonary hypertension (ESPAP <25) (N=21) is 0.46 ± 0.36. Difference between 2 groups is statistically significant (p=0.034).

Regarding Echo findings, 14 thalassemic cases (35%) had mild pulmonary hypertension while 6 cases (15%) had moderate pulmonary hypertension. None of our studied patients had systolic dysfunction (Ejection fraction in all patients was > 55%) as presented in Table 5. We found that PDMP was higher in patients with pulmonary hypertension with significant difference (p-value = 0.034) (Figure 1).

5. Discussion

It has been reported that novel visions into the association between defective hemoglobin synthesis, RBC perturbation, and pathophysiological complications can be detected by assessment of serum level of circulating MPs in thalassemia which is a simple and consistent method (Pattanapanyasat et al., 2004). In the current study, incidence of β-thalassemia was highest among consanguineous marriages (72.5%) compared to un-related ones. Which was reported by other studies (Khan et al., 2015; Yaman et al., 2015).

As expected in CBC of thalassemic patients, our cases had significantly lower mean Hb level compared to healthy controls (6.2 Vs 10.6). On the other hand, mean Hb level of healthy controls was still low according to reference range of normal Hb level for age and sex (The University of Iowa, Department of Pathology, "Pediatric Reference Ranges - Laboratory Services Handbook", 2017); where 48% of the controls had mild anemia which appeared to be of microcytic hypochromic type.

WBCs count is usually elevated in β-thalassemia major; this is due, in part, to miscounting the many nucleated RBCs as leukocytes. Leukocytosis is usually present, even after excluding the nucleated RBCs. This could be a part of hemolysis or post-splenectomy sequelae. Therefore, it wasn't surprising to find higher mean of WBCs count among our studied thalassemic cases compared to healthy controls (12.7 Vs 8.8).

By measurement of serum level of PDMPs among our different study groups we found that the mean level of PDMPs was significantly higher among cases compared to healthy controls (1.85 Vs 0.71) with p-value <0.001. This was consistent with other studies that reported that PDMPS were significantly higher in β-thalassemia major patients than healthy controls ($p < 0.001$) (Tantawy et al., 2013; Agouti et al., 2015).

In this study, PDMPs levels were significantly increased in β-thalassemia major patients with serum ferritin >2500 ng/ml, suggesting a relationship between PDMPs and iron overload. These results were in agreement with other studies (Tantawy et al., 2013; Elsayh et al., 2013). Thirty percent of our studied thalassemic patients (12/40) underwent splenectomy. We found that splenectomized patients had significantly higher age (mean 11.3 Vs 7.9), longer disease duration (mean 10.8 Vs 6.6), significantly higher platelet count (mean 435.5 vs 297.4) and higher PDMP (2.22 Vs 0.73) compared with non-splenectomized counterparts. Splenectomy could result in the increased amounts of MPs in blood circulation. MPs, especially from splenectomized patients, had high efficiency to form prothombinase complexes (Chaichompoo et al, 2012).

One of the leading cause of mortality and morbidity in β-thalassemia major is cardiovascular involvement (Aessopos et al., 2007). In β-thalassemia major pulmonary hypertension seems to happen more commonly and at an early stage of cardiac involvement (Du et al., 1997). All the studied patients were clinically asymptomatic for cardiopulmonary abnormalities. Screening for the risk of pulmonary hypertension was performed by the non-invasive M-mode Doppler echocardiography with different modalities to evaluate left ventricular function, pulmonary artery pressure, and tricuspid regurgitant jet velocity (TRV). Echocardiography can be used as a non-invasive method to estimate pulmonary pressures by measuring the TRV (Gladwin et al., 2008). In the present study, pulmonary hypertension was found in 50% (20/40) of our patients. Fourteen patients had mild pulmonary hypertension while only six cases had moderate pulmonary hypertension. These data are consistent with the data reported by

other groups (Dedeoglu & Bornaun, 2017; Tantawy et al., (2013). We also found that pulmonary hypertension had higher mean value in splenectomized cases which was reported by another study comparing pulmonary hypertension between splenectomized and non splenectomized cases (Tantawy et al., 2013).

None of our studied patients had systolic dysfunction (EF in all patients was > 50% ranging between 60-83%). These findings were in agreement with other studies (Abhay et al., 2016; Eshaqhosseni et al., 2014). The hemodynamic effects associated with anemia assisted to maintain average ejection fraction and myocardial fiber shortening. This may explain the failure to detect impaired ventricular systolic function in thalassemia patients (Eshaqhosseni et al., 2014).

We found that PDMP was significantly higher in patients with pulmonary hypertension than patients with normal pulmonary artery pressure. This was consistent with Ogawa et al., (2012) who reported that PDMP may be involved in the pathogenesis of pulmonary hypertension due to the high levels found in those patients. Also Kqiku et al., (2011) reported that the overall fraction of MPs was higher in PH patients.

None of our study cases experienced any thromboembolic events (TEE). However, Borgna et al., (1998) surveyed nine italian pediatric thalassemia centers, observing that 4% of the 683 patients with TM and 9.6% of the 52 patients with TI had experienced a TEE.23 Age above 20 years, splenectomy, family history of TEE, and previous TEE were identified as the main risk factors for thrombosis in TI. Moreover, patients receiving aspirin therapy had a significantly lower rate of recurrent TEE.

PDMPs levels were significantly elevated in thalassemic patients compared to healthy controls. Fifty percent of our cases had mild to moderate pulmonary hypertension. PDMP was significantly higher in patients with pulmonary hypertension. We suggest that increased PDMPs may be implicated in vascular dysfunction and pulmonary hypertension risk observed in thalassemia patients. Assessment of PDMPs might provide efficacy for early detection of cardiovascular abnormalities and monitoring the biological efficiency of chelation therapy.

We showed that Platelet-derived microparticles (PDMPs) is implicated in vascular dysfunction and the risk of pulmonary hypertension in thalassemia patients. Their quantification could provide utility for early detection of cardiovascular abnormalities.

Corresponding author:
Mohammed Mansour Abbas Eid, M.D. and Ph.D.,
Lecturer,
Department of Clinical Pathology,
Faculty of Medicine, Fayoum University
Fayoum City, PO Box 63514, Egypt
Tel: +2-127-408-3431
Fax: +2-84-630-2350
E-mail: mma21@fayoum.edu.eg

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