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Early Predictors of Thromboembolism in Pediatric Cardiac Patients

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Abstract

Background: The precise mechanisms of the increased incidence of hemostatic abnormalities in congenital heart disease (CHD) have not been determined. The aim of the study was to evaluate some indicators of activation of platelets and vascular endothelial cells in patients with CHD.

Subjects and methods: This work was carried out on 40 patients with acyanotic congenital heart disease (ACHD), 40 patients with cyanotic heart disease (CCHD) and 30 healthy children as a control group, aged between 1-10 years. All subjected were subjected to full clinical examination, CBC, oxygen saturation, echocardiography, bleeding and coagulation times, PT, PTT, FDPs, soluble plasma selectin (sP- selectin), E-selectin and platelets factor 4 (PF4).

Results: There was significant elevation in sP- selectin (128.9±42.44ng/dl), E-selectin (9461.5±1701.24Pg/ml) and PF4 (54.99±25.54ng/ml) levels in children with CCHD as compared to ACHD (sP-selectin 80.1±13.21ng/ml, E- selectin 7969.6±2127.5Pg/ml and PF4 was 21±7.94ng/ml,) and significant increase in both groups when compared to control group (P- selectin 27.83±9.73ng/ml, E- selectin 6750±3204 and Pg/ml PF4 8.1±4.7ng/ml). There was significant negative correlation between oxygen saturation and plasma P-selectin (r -0.865) and PF4 (r -0.792) in CCHD.

Conclusion: There is significant rise in sP-selectin, E- selectin and PF4 in patients with CHD as compared with normal healthy children; that was significantly higher in CCHD as compared with ACHD.

Keywords: Platelet; Endothelial; Selectins; PF4; CHD

Introduction

The incidence of congenital heart disease (CHD) is approximately six to eight in 1,000 live births [1]. In patients with cyanotic congenital heart disease (CCHD) with hypoxemia, secondary erythrocytosis, high-shear stress of the vessel wall and platelet surface in association with blood hyperviscosity cause chronic endothelial dysfunction as well as platelet activation, that favour thrombogenesis in the microcirculation and increase the risk of thromboembolism [2,3].

The precise mechanisms of the increased incidence of thromboembolism in patients with CCHD have not yet been determined, but endothelial dysfunction, hemostatic abnormalities and platelet activation may be underlying factors causing hypercoagulability and thromboembolism [4].

P-selectin is an adhesion molecule found in the secretory granules of platelets and Weibel-Palade bodies of endothelial cells, and is mobilized to the plasma membrane on activation [5]. Activated platelets expressing P-selectin on the surface release their granule contents, facilitating the adhesion of platelets and neutrophils to the endothelium and causing platelet aggregation and enlargement of thrombi through recruitment of leucocytes and platelets. Thus, P-selectin expressed on platelets is likely to play an important role in thrombus formation [6,7].

In humans, E-selectin is encoded by the Sele gene. Its C-type lectin domain, EGF-like, SCR repeats, and trans membrane domains are each encoded by separate exons, whereas the E-selectin cytosolic domain derives from two exons. The E-selectin locus flanks the L-selectin locus on chromosome 1 [8].

Different from P-selectin, which is stored in vesicles called Weibel-Palade bodies, E-selectin is not stored in the cell and has to be transcribed, translated, and transported to the cell surface. The production of E-selectin is stimulated by the expression of P-selectin which is stimulated by tumor necrosis factor α (TNFα), and it can also be stimulated by interleukin-1 (IL-1) and lipopolysaccharide (LPS) [9].

Platelet factor-4 is a 70-amino acid protein that is released from the alpha-granules of activated platelets and binds with high affinity to heparin. Its major physiologic role appears to be neutralization of heparin-like molecules on the endothelial surface of blood vessels, thereby inhibiting local antithrombin III activity and promoting coagulation. As a strong chemo-attractant for neutrophils and fibroblasts, PF4 probably has a role in inflammation and wound repair [10]. Aim of the study was to evaluate some indicators of activation of platelets and vascular endothelial cells in patients with CHD and their correlation with the hemostatic disorders in these patients.

Subjects and Methods

Design of the study and setting

This prospective study was carried out after approval from Research Ethical Committee Centre of Zagazig University Hospital and obtaining an informed oral or written consents from parents of included patients and controls over a period of twenty four months from August 2016 to August 2017 ,on 80 children with congenital heart disease; forty children with acyanotic CHD and 40 children with cyanotic CHD selected from Pediatric Department of AUH as tertiary care hospitals .Thirty apparently healthy children matched for age and sex served as control group in this study.

Inclusion criteria

Children with congenital heart disease from Pediatric Departments of Zagazig University Hospital who approved participation in the study.

Exclusion criteria

Children with congenital heart disease receiving any drug affecting hemostasis as antiplatelets or anticoagulants. Children with congenital heart disease with other organ disease as hepatic or renal diseases that can affect hemostasis. CHD with infection. CHD complicated with heart failure. Children were underwent complete history taking, clinical examination and the following investigations; Chest X-ray, ECG, Echocardiography, SaO_2, CBC, Bleeding time and clotting time, PT and a PTT and FDP. Patients with pulmonary hypertension were under gone cardiac catheterization for the evaluation of their pulmonary vascular resistance.

Research investigations

1-Plasma level of sP-selectin assay: by quantitative sandwich immunoassay technique [11].

2- Plasma level of E-selectin assay: by the quantitative sandwich immunoassay technique [12].

3-Plasma level of platelet factor 4(PF4) assay: by Zymutest PF4 which is a sandwich ELISA designed with affinity purified rabbit polyclonal antibodies specific for human PF4 [13].

Statistical Analysis

The collected data were tabulated and analyzed using Statistical Package for Social Science (SPSS version 16). Categorical data were presented as number and percentages and comparison between them in studied groups was performed with student t-test while quantitative data were expressed as mean±standard deviation. Chi square test (X^2) was done for qualitative data, linear Correlation Coefficient [r] was used for correlations between different studied variables. The accepted level of significance in this work was stated at 0.05. P value>0.05 is non-significant P<0.05 is significant. P≤0.001 is highly significant [14].

Results

This study has been carried out on 80 CHD patients; which were classified into 2 groups; 20 ACHD including 18 males and 22 females, their age ranged between 1 and 10 years (5.79±2.98). The cardiac diagnosis of this group of patients was 20 patients with VSD, 12 patients with ASD and 8 patients with PDA. The other group included 20 CCHD included 24 males and 16 females, their age ranged between 1.5 and 8 years (4.64±1.7). The cardiac diagnosis of this group of patients was 18 patients with fallot tetrology, 14 patients with transverse position of the great arteries and 8 patients with double outlet right ventricle. Ten normal children matched for age and sex were taken as control group.

Table 1: Shows statistical analysis of PLT count (x103/dl) PT, aPTT (in seconds) and FDP (mcg/ml) in all studied groups.

		ACHD	CCHD	Control	F. Test			T Test
					Value	p. Values		
PLT (x103/dl)	Range	180-475	168-306	324-449	18.791	P=0.001	P1	0.001
							P2	0.031
	Mean+ SD	337.1+102.65	235.57+47.79	394.5+43.19			P3	0.001
HCT (gm%)	Range	28.2-45.5	34.5-76.9	29.2-45.9	19.914	P=0.001	P1	0.749
							P2	0.001
	Mean± SD	38.49±5.74	56.72±14.56	37.2±6.65			P3	0.001
PT in Seconds	Range	10-16.5	10-20.20	12-14	4.778	P=0.013	P1	0.001
							P2	0.978
	Mean +SD	12.94+2.34	15.45+3.55	13.17+0.713			P3	0.021

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aPTT in Seconds	**Range**	25-45.6	27-81	25-40	8.999	P=0.001	P1	0.003
							P2	0.822
	Mean+ SD	33.53+7.42	48.97+17.96	5.74+1.81			P3	0.001
FDP mcg/ml	**Range**	1-10.1	1.5-12	1-10.5	0.536	P=0.589	P1	0.398
							P2	0.44
	Mean+ SD	6.36+3.11	6.9+3.38	5.62+3.17			P3	0.927

HCT: Hematocrite values

PLT: Platelet counts.

PT: Prothrombin time.

aPTT: Activated partial thromboplastin time.

FDP: Fibrinogen degradation products.

CCHD: Cyanotic congenital heart disease

ACHD: Acyanotic congenital heart disease

*P is P value of F test comparing between CCHD, CCHD &Control groups.

*P1 is P value of test comparing between CCHD & Control

*P 2 is P value of t test comparing between ACHD & Control

*P3 is P value of t test comparing between CCHD & ACHD].

This Table 1 showed significant decline in PLT count ($x10^3$/dl) in CCHD than in ACHD than in controls. There was significant prolongation in a PTT and PT (in seconds) in CCHD than ACHD and controls but with no significant difference between them in ACHD and controls. There is no significant difference in FDPs (mcg/ml) between all studied groups.

Table 2: Indicators of vascular endothelial and platelet activation (sP-selectin (ng/ml), E- selectin (pgm/ml) and PF4 (ng/ml)) in all studied groups.

		ACHD	CCHD	Control		F. Test	p. Value	
sP-selectin (ng/ml)	**Range**	55-100	70-204	18-45	42.454	P=0.001	P_1	0.001
							P_2	0.001
	Mean +SD	80.1+13.21	128.9+42.44	27.83+9.73			P_3	0.001
E-Selectin (pgm/ml)	**Range**	2750-11750	4750-11000	2500 - 11500		P=0.001	P_1	0.001
							P_2	0.001
	Mean +SD	7969.6±2127.5	9461.5±1701.24	6750±3204			P_3	0.25
Platelet factor 4 (ng/ml)	**Range**	10-34	15.60-91	1-16	31.172	P=0.001	P_1	0.001
							P_2	0.049
	Mean +SD	21+7.94	54.99+25.54	8.1+4.70			P_3	0.001

CCHD: Cyanotic congenital heart disease

ACHD: Acyanotic congenital heart disease.

*P is P value of F test comparing between CCHD, CCHD & Control groups.

*P1 is P value of t test comparing between CCHD & Control

*P2 is P value of t test comparing between ACHD & Control

*P3 is P value of t test comparing between CCHD & ACHD

This Table 2 showed that there was a statistically significant rise in plasma sP-selectin (ng/ml) in CCHD than in ACHD and in controls and significant rise in ACHD than in controls (Figure 1). There is significant rise in E-selectin (pg/ml) in CCHD than in ACHD and in control and significant rise in ACHD than in control (Figure 2). There is also significant rise in plasma PF4 (ng/ml) in CCHD than in both ACHD and control and significant rise in ACHD than in controls (Figure 3).

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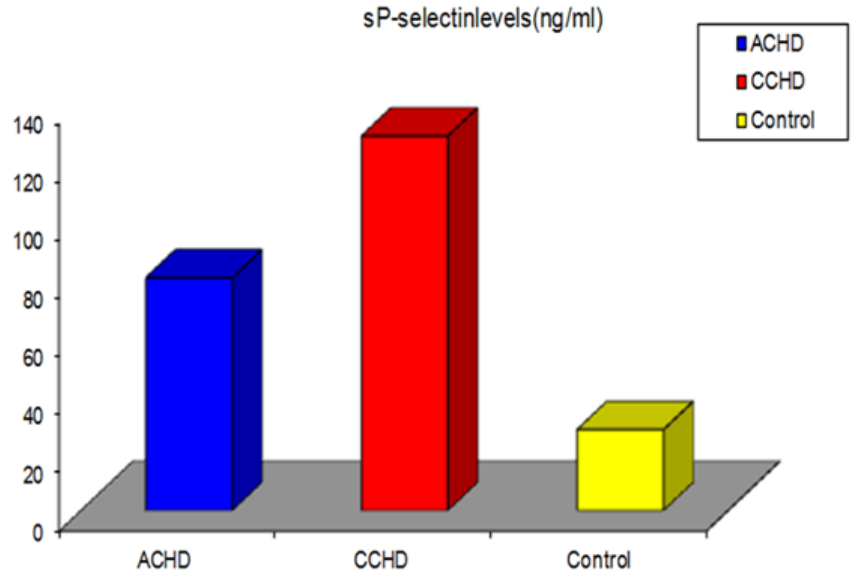

Figure 1: Illustrate plasma sP-selectin levels (ng/ml) in all studied groups.

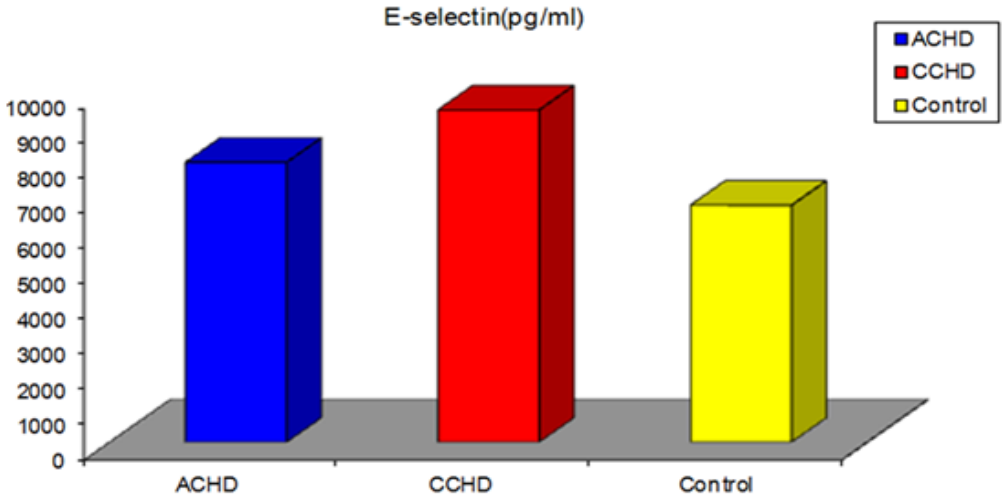

Figure 2: Illustrate plasma E-selectin levels (pg/ml) in all studied groups.

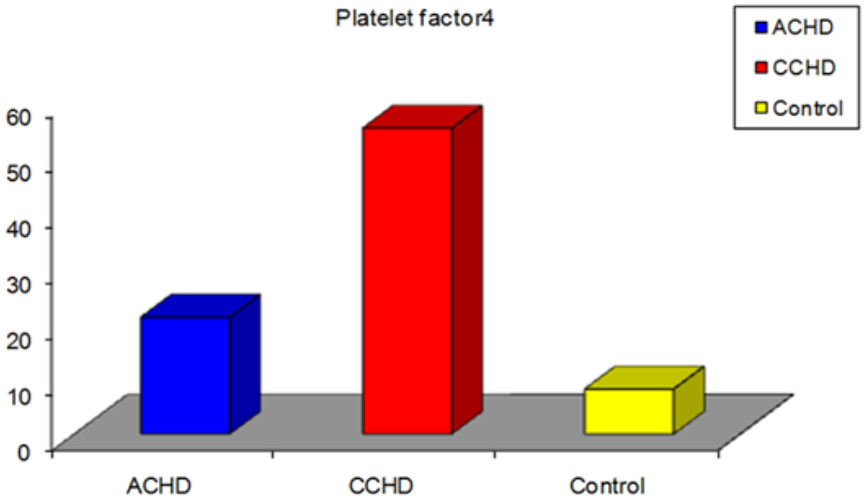

Figure 3: Illustrate platelet factor 4(ng/ml) in all studied groups.

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Table 3: Relation of E- selectin in pulmonary vascular resistance (PVRi) in the studied acyanotic CHD group.

	PVRi				p-Value
	PVRi <2(N=22)		PVRi >2(N=18)		
	Mean	SD±	Mean	SD±	
Serum E-selectin (pg/ml) Range (7969.6±2127.5)	8875	1671.45	7290.63	2276.19	0.17

ACHD: acyanotic congenital heart disease.

*P is P value of F test comparing between CCHD, CCHD &Control groups.

*P1 is P value of t test comparing between CCHD & Control

*P 2 is P value of t test comparing between ACHD & Control

*P3 is P value of t test comparing between CCHD & ACHD.

Table 3 showed that no significant difference in the studied 18 patients who were diagnosed as ACHD with increased pulmonary vascular resistance (PVRi) (<2 wood unit) in comparison to the studied 22 patients who were diagnosed as ACHD with normal PVRi (<2 wood unit) (p>0.05). This Table 4 showed that there were statistically significant negative correlations between SaO2 with plasma sP-selectin & platelet factor 4 in CCHD.

There were statistically significant positive correlations between plasma sP-selectin and both HCT and platelet counts in the two studied CHD groups (Table 4). There were also statistically significant positive correlations between platelet factor 4 and both HCT and platelet counts in the two studied CHD groups (Table 4). Table 5 showed that no significant correlations between the level of E- Selectin and the echocardiographic findings of our studied patients with ACHD.

Table 4: Correlations between plasma sP-selectin, platelet factor 4 and E-selectin and some of the studied laboratory variables in our cyanotic and acyanotic CHD patients.

	SaO_2				HCT Value				PLT. Count			
	CCHD		ACHD		CCHD		ACHD		CCHD		ACHD	
	r	P value	r	P value	r	P value	r	P value	r	P value	r	P value
sP selectin	-0.865	0.001	-0.207	0.381	0.785	0.001	0.448	0.001	-0.857	0.001	-0.776	0.001
E-selectin	0.409	0.166	0.085	0.773	-0.161	0.6	0.271	0.348	-0.51	0.868	-0.418	0.137
PF4	-0.792	0.001	-0.392	0.087	0.754	0.001	0.708	0.001	-0.850	0.001	-0.787	0.001

PF4: Platelet factor 4.

SaO_2: Oxygen saturation.

HCT: Hematocrite values

PLT: Platelet counts.

CCHD: Cyanotic congenital heart disease

ACHD: Acyanotic congenital heart disease.

r: Correlation coefficient.

Table 5: Correlations of the studied E- selectin with Echocardiographic findings in acyanotic CHD group.

		mPAP	PASP	QP- QS	PVRi
Serum E- Selectin	r	-0.092	-0.47	0.154	-0.323
	p	0.753	0.09	0.6	0.26
	Number of patients	40	40	40	40

ACHD: Acyanotic congenital heart disease

mPAP: Mean pulmonary arterial pressure

PASP: Pulmonary arterial systolic pressure

PVRi: Pulmonary vascular resistance

r: Correleation coefficient.

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Discussion

The current study showed a high statistically significant reduction in platelets of CCHD patients as compared to ACHD and control group observation was reported by Temp et al. [15]; Lill et al. [16], El Sedawy [17] and Horigome et al. [18] who reported a very high significant decrease in platelets count in CCHD. They hypothesized many reasons for thrombocytopenia in patients with CCHD such as increased PLT destruction, increase PLT activation, shortened life span of the platelets or decreased production of platelets as megakaryocytes escape fragmentation in plethoric lungs associating CHD with right to left shunt and bone marrow depression secondary to chronic hypoxemia. The lower the systemic arterial oxygen saturation, the higher the HCT, and the lower the platelet count. These relations were previously reported and were confirmed in their study.

The current study showed that plasma levels of Platelet Factor 4 were significantly elevated in both CHD groups than in control and were significantly elevated in CCHD more than ACHD. Horigome et al. [7] showed that the plasma levels of PF4, contents of the alpha-granule and specific markers of platelet activation were significantly elevated in CCHD group compared with ACHD group. Also Rosove et al. [19] reported that Plasma PF4 levels in patients with CCHD were higher than in normal subjects. Kroll et al. [20] suggested marked activation of the platelet system in patients with CCHD as shown by a significant increase in the level of biochemical markers of hemostasis such as platelet factor 4, thromboxane and β- thromboglobulin.

In the current study significant elevation in plasma s P-selectin levels were found in children with CCHD and ACHD compared with healthy controls, and significant elevation in CCHD than ACHD was reported. In agreement with the current study Horigome et al. [8] demonstrated that plasma levels of soluble P-selectin were significantly elevated in CCHD group compared with ACHD group. In agreement with the current study Ismail and Youssef reported that the values of P-selectin showed significant increase in both CCHD and ACHD groups compared with healthy children However, the elevation of P-selectin was more evident in patients with CCHD than ACHD group [21].

Kajimoto et al. [22] showed that P-selectin expression on the platelets is elevated (which is soon lost into the circulation as s P-selectin), indicating that platelet activation occurs in CCHD. Although many patients were receiving an antiplatelet drug (aspirin or ticlopidine) or a combination of an antiplatelet and an anticoagulant drug, platelet P-selectin was elevated in many patients [22].

Also Levin et al. [4] also failed to demonstrate an increase in platelet surface P-selectin. This discrepancy might be derived from the phenomenon that P-selectin is rapidly mobilized to the platelet surface on activation and is soon lost into the circulation. Endothelium origin of plasma P-selectin cannot be excluded in the current study [4].

Frijns et al. [23] reported that the correlation found between sP-selectin and sE-selectin suggests that part of sP-selectin originates from endothelial cells and the remainder from platelets, so increased levels of sP-selectin reflect activation of both vascular endothelial cells and platelets [23].

Our study showed no correlation of the level of E-Selectin with the different echocardiographic findings in the ACHD group. Also, there was no significant difference of its level in patients with increased pulmonary vascular resistance when compared with those with normal pulmonary vascular resistance.

Smadja et al. [24] reported that, among a large panel of biomarkers reflecting endothelial activation, regeneration, and injury, the high circulating endothelial cells levels was proved to be the only marker allowing discrimination between reversible and irreversible PAH secondary to CHD and the levels of soluble markers indicating endothelial activation including E-selectin were not significantly different between reversible and irreversible PAH patients [24].

Conclusion

Variable degrees of thrombohemmorrhage and coagulopathy represent a dilemma in patients with CHD. Chronic hypoxemia in patients with CCHD leads to secondary erythrocytosis and platelet and endothelial cell activation that favoring thrombogenesis and increases the liability for thrombosis.

The serum levels of sP-selectin and platelet factor 4 are more reliable indicators than the level of E-selectin in detection of hemostatic disorder in children with congenital heart diseases.

Recommendations

From this current study we recommend that:

a) Early surgical correction of CHD to avoid hypoxia with subsequent hyperviscosity and increased shear stress in CCHD and hemodynamic disturbances with accelerated and turbulent flow in ACHD which lead to platelet and vascular endothelial cell activation with increased risk of thromboembolic consequences.

b) Hematocrit level and platelet count should be interpreted in relation to one another in the regular follow up of patients with CCHD to predict conditions predisposing to thrombosis. Prospective studies are needed to assess the potential impact of platelet and vascular endothelial cell activation markers such as sP-selectin and platelet factor 4 on the clinical outcome of those patients and response to therapy. Moreover further analysis including larger number of children would help to verify our findings.

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