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ORIGINAL ARTICLE

Role of Thrombophilic Mutations in Childhood Cardiac and Great Vessel Thrombosis

Barış Malbora[1], Namık Özbek[2], Zekai Avcı[2], Hasibe Verdi[3], Bülent Alioğlu[2], Birgül Varan[4], Fatma Belgin Ataç[3]

[1]Department of Pediatric Hematology, Dr. Sami Ulus Maternity and Children Training and Research Hospital, Departments of [2]Pediatric Hematology, [3]Medical Biology and [4]Pediatric Cardiology, Baskent University Faculty of Medicine, Ankara, Turkey

Abstract:

Objective: This study was conducted to analyze the thrombophilic mutations of children with cardiac and great vessel thrombosis diagnosed in our hospital and investigate the risk factors.Methods: The clinical and laboratory findings of 41 children having cardiac and/or great vessel thrombosis between 1997 and 2009 were retrospectively analyzed. Results: All patients with cardiac thrombosis had an underlying associated clinical condition. The frequency of cardiac thrombosis was highest in children older than one year. Patients with congenital heart disease were the largest group (53.7%). Acquired risk factors were identified in 32 of the 41 patients. There was a significant relationship between right atrial thrombosis and wearing a central venous catheter, between right atrial thrombosis and systemic infection, between right ventricle thrombosis and angiographic procedure, and between right ventricle thrombosis and systemic infection. 33 of 41 patients (80.5%) who were examined had at least one thrombophilic mutation. Conclusion: Our study implies not only the underlying disorders but the factor V Leiden and prothrombin G20210A mutations are the important predisposing molecular risk factors for cardiac thrombosis.

Keywords: *Congenital heart disease, cardiac thrombosis, great vessel thrombosis, thrombophilic mutations, children,*
Corresponding author: *Baris MALBORA, MD; Dr. Sami Ulus Maternity and Children Training and Research Hospital, Department of Pediatric Hematology, Altındağ, Ankara, Turkey*
Tel: +90 312 3056182
E-mail: barismalbora@gmail.com

Introduction

Thrombosis is a multifactorial disorder that affects people of all ages. However, the incidence is quite low in children compared to adults (0.07 per 10,000 vs 2.5–5%) (1). Both genetic and acquired risk factors are responsible for thrombosis in children (1,2). Due to common use of cross-sectional echocardiography, cardiac thrombosis (CT) in children is diagnosed more frequently today (3). Atrial fibrillation, left ventricular dysfunction, valvular heart disease, antiphospholipid antibodies, congenital heart disease (CHD) and CHD operations are some factors that may cause CT. In addition to these factors, genetic predisposition to thrombosis may explain why some of these patients develop CT (4).

In this study, we planned to analyze some thrombophilic mutations and single nucleotide polymorphisms of the genes encoding

coagulation factors in children who had cardiac and/or great vessel thrombosis (CGVT).

Materıals And Methods

Subjects

Great vessels are inferior vena cava, aorta, superior vena cava, portal vein, pulmonary vein and pulmonary artery. We included in 143 patients who were diagnosed with thrombosis in our institution from January 1997 to June 2009. Out of 143 patients, 41 patients had CGVT (age range 0-18 years). Thrombosis has been defined as the thrombi within heart chambers and/or great vessels. Data of the patients were obtained from their hospital medical records. Comprehensive clinical data forms, included demographic data, location of thrombus, diagnostic test(s) used for detection of thrombus, associated conditions (CHD, dilated cardiomyopathy, renal disease, malignancy, systemic infection, respiratory distress syndrome, liver disease and others), genetic and acquired risk factors were investigated for each child. All thromboses were confirmed by the results of an objective radiographic test.

Laboratory tests

The results of the following laboratory tests, which were performed at the time of diagnosis, were recorded for each patient. A daily calibrated automatic hemocytometer (Cell Dyn 3700, Abbott, Abbott Park, IL, USA) was used to perform a complete blood cell count. Coagulation tests were performed with a commercially available kit (the IL-200, Instrumentation Laboratory, Italy, was used for the patients treated before 2000; and the Asserachrom, Diagnostica Stago, Asnieres, France, was used for the patients treated after 2000). Fibrinogen level was determined from the platelet-poor plasma. Lupus anticoagulant in plasma was measured via a coagulometric technique with a commercially available kit (Stago, France). The levels of anti-cardiolipin antibodies (ACA), immunoglobulin (Ig) M, and IgG in serum or plasma were measured with an enzyme-linked immunosorbent assay kit (Dynex DSX™ Four-Plate Automated ELISA Processing System, USA). The level of lipoprotein (a) (Lp(a)) was measured via an immunoturbidimetric technique with a Roche Clinic Chemistry Analyser in serum or plasma. The homocysteine level was measured by a fluorescence polarization immunoassay technique with an Axsym (Abbott Laboratories, Abbott Park, IL, United States) in serum or plasma. Quantitative cholesterol and triglyceride levels were measured in serum via an enzymatic technique with a Roche Moduler (Basel, Switzerland).

Laboratory values

Polycythemia was defined as a hematocrit level higher than 65% for neonates and higher than 55% for all other subjects. Thrombocytosis was defined as a platelet count higher than 450 × 109/L, and hyperfibrinogenemia was defined as a fibrinogen level higher than 400 mg/dL. Age dependent values within the normal range were used for hemoglobin and hematocrit levels and erythrocyte volume (5). The upper limits of the following parameters were as follows: ACA IgM, 19 MPL U/L; ACA IgG, 19 GPL U/L; lupus anticoagulant, 60 seconds; total cholesterol level, 200 mg/dL; and total triglyceride level, 150 mg/dL. Levels higher than 30 mg/dL for Lp(a) was defined as elevated. We determined the homocysteine upper limits for ages referring to the study of Altuntas et al (6) due to variability of these.

Genotyping

After obtaining the informed consent form from the families of the children, a total of 2 ml venous blood was drawn into tubes containing EDTA for genotyping. Genomic DNA was prepared from leukocyte pellets by sodium dodecyl sulphate lysis, ammonium acetate extraction and ethanol precipitation (7). The primers and polymerase chain reaction (PCR) conditions were performed as previously described (8-11).

In order to analyze the factor V Leiden (FVL) G1691A mutation, the 241bp digested PCR

Table 1. Demographic data and associated clinical conditions of patients with cardiac thrombosis

Age median (range)	Sex (male/female)	Underlying disease n (%)						
		CHD 22 (53.7%)		CMP	CRF	Malignancy	Others	Healthy
		c-CHD	ac-CHD					
48 month (4 w-18 y)	27/14	16 (39%)	6 (14.6%)	5 (12.2%)	5 (12.2%)	3 (7.3%)	5 (12.2%)	3 (7.3%)

CHD, congenital heart disease; c-CHD, cyanotic congenital heart disease; ac-CHD, acyanotic congenital heart disease; CMP, cardiomyopathy; CRF, chronic renal failure; w, week; y, year

products were separated by electrophoresis in 12% PAGE. In the presence of A allele, the PCR product (241bp) was cut into two fragments of 209 bp and 32 bp by HindIII digestion. The ethidium bromide stained gel showed a non-digested band of 241 bp for G allele.

Factor V Cambridge (A1090G), the GG homozygote one fragment of 228 bp did not splice with BstNI restriction enzyme due to the substitution of A by G. However the AA homozygote produced two fragments at 162bp and 66bp.

Factor V A1299G mutation the 877 bp PCR product was cut with RsaI. The uncut product 877 bp shows the presence of A allel, if the PCR product was cut into two fragments as 476 bp and 401 bp, it reveals the G allele for.

The PCR product of 345 bp was digested with HindIII enzyme according to the manufacturer's instruction for the analysis of the prothrombin (Prt) G20210A mutation. The 20210G allele lacks of the Hind III site. However the 20210A variant has two bands corresponding to both 322bp and 23 bp.

We routinely analyze the methylene tetrahydrofolate reductase (MTHFR) mutations since these are frequently observed in our population (10). For the analysis of MTHFR C677T 198 bp PCR product was cut with Hinf1. The uncut product (198 bp) shows the presence of C allele whereas the 175 and 23 bp it reveals the T allele. 163 bp PCR product was digested with MbOII enzyme for genotyping of MTHFR A1298C mutation. Wild type produced 5 fragments of 56, 31, 30, 28, 18 bp while the C allel reveals 4 fragments of 84, 31, 30, 18 bp. PCR product was digested with TaqI enzyme for MTHFR T1317C mutation. The 1317 C has two bands corresponding to both 202 bp and 25 bp however the 1317 T allele lacks the TaqI site.

In order to genotype plasminogen activator inhibitor (PAI)-1 4G/5G polymorphism a 98 bp PCR product was cut with Bsl I. The two fragments as 77 and 22 bp it reveals the 5G allele and the uncut corresponds to the G allele.

Ethics

The present study was performed in accordance with the ethical standards set forth in an updated version of the 1964 Declaration of Helsinki and approved by the medical ethics committee of Baskent University, Ankara, Turkey. All subjects included in this study gave informed consent form.

Results

Cardiac and great vessel thrombosis has been diagnosed in 41 children (27 male, 14 female; median age 48 months; range 4 weeks-18 years) during the study period (Table 1).

Molecular analysis has been possible in all of these children. Of 41 patients, 20 had isolated CT, 9 had isolated great vessel thrombosis, and

Table 3. Congenital, laboratory and acquired risk factors of patients with cardiac thrombosis

Congenital (n=33; 80.5%)	**Total FV mutations (n=17; 41.5%)**	**FV G1691A**	G1691G	34 (83%)
			G1691A	6 (14.6%)
			A1691A	1 (2.4%)
		FV A1090G (Cambridge)	A1090A	37 (90.2%)
			A1090G	2 (4.9%)
			G1090G	2 (4.9%)
		FV His1299Arg	His 1299 His	31 (75.6%)
			His 1299 Arg	9 (22%)
			Arg 1299 Arg	1 (2.4%)
	Prt G20210A (n=2; 4.8%)		G20210G	39 (95.2%)
			G20210A	2 (4.8%)
			A20210A	None
	Total MTHFR mutations (n=27; 65.9%)	**MTHFR C677T**	C677C	30 (73.2%)
			C677T	9 (21.9%)
			T677T	2 (4.9%)
		MTHFR A1298C	A1298A	21 (51.3%)
			A1298C	14 (34.1%)
			C1298C	6 (14.6%)
		MTHFR T1317C	T1317T	38 (92.7%)
			T1317C	3 (7.3%)
			C1317C	None
	PAI-1 4G/5G (n=24; 58.5%)		4G/4G	12 (29.3%)
			4G/5G	12 (29.3%)
			5G/5G	17 (41.4%)
Laboratory	**Total (n=24; 58.5%)**		Hyperfibrinogenemia	8 (19.5%)
			Polycytemia	5 (12.2%)
			Lupus anticoagulant (+)	5 (12.2%)
			Thrombocytosis	4 (9.8%)
			ACA (+)	4 (9.8%)
			Lipoprotein(a) (↑)	3 (7.3%)
			Hypercholesterolemia/ hypertriglyceridemia	2 (4.9%)
			Hyperhomocysteinemia	22 (53.7%)
			None	17 (41.5%)
Acquired	**Total (n=32; 78%)**		Surgery	19 (46.3%)
			Catheter	18 (43.9%)
			Infection	8 (19.5%)
			None	9 (21.9%)

ACA, anticardiolipin antibody; FV, Factor V; MTHFR, methylene tetrahydrofolate reductase; Prt, Prothrombin, PAI-1, plasminogen activator inhibitor-1.

Table 2. Anatomic sites of thrombosis of patients with cardiac thrombosis

	Site of thrombosis	n (%)
Cardiac and/or great vessel thrombosis	Right atrium	18 (43.9%)
	Right ventricle	7 (17.1%)
	Left atrium	4 (9.8%)
	Left ventricle	5 (12.2%)
	Superior vena cava	5 (12.2%)
	İnferior vena cava	5 (12.2%)
	Pulmonary artery	8 (19.5%)
	Aorta	4 (9.8%)
	Blalock-Taussig shunt	3 (7.3%)
Extra-cardiac thrombosis	Extremity vein	6 (14.6%)
	Extremity artery	6 (14.6%)
	Abdominal vein	6 (14.6%)
	None	28 (68.3%)

12 had another thrombosis site in addition to cardiac and/or great vessel thrombosis. During the study period 143 patients were diagnosed with thrombosis in our hospital including CGVT. Therefore, the rate of CGVT was approximately 29% and CT was 21.7% in our study group. Fifty-nine CGVT anatomic sites were detected in 41 patients who were enrolled in our study (Table 2).

Patients with CHD were the largest group (n=22; 53.7%) (Table1). Among them, 16 patients had cyanotic CHD. Associated clinical conditions showed that table 1. Among all patients, 16 had associated clinical condition(s) other than CHD. Acquired risk factors were identified in 32 (78%) of the 41 patients, 16 of which had more than one (39%) risk factor (Table 3). Development of right heart thrombosis was significantly higher than the left heart thrombosis in patients who had acquired risk factors. Cardiac surgery was most frequent operation in the study subjects. Our study also revealed three significant relationships; between RV thrombosis and wearing a central venous catheter, RA thrombosis and systemic infection, and RV thrombosis and systemic infection.

Laboratory tests

The laboratory test results are summarized in table 3. Abnormal laboratory result(s) were detected in 33 (80.5%) of 41 patients. However, 15 of these 33 patients had more than one laboratory abnormality.

Congenital risk factors

All of 41 patients were assessed for thrombophilic mutation screening. Thirty-three (80.5%) had at least one thrombophilic mutation and/or PAI-1 4G allele. Twenty-six (63.4%) patients had more than one genetic mutation and/or PAI-1 4G allele. Overall 12 homozygous and 45 heterozygous variations were detected (Table 3).

Discussion

The dramatic increase in childhood venous thromboembolism has been shown in the last decade (12). The incidence of CT, which is relatively rare in children, has been increasing in recent years. However, limited data on childhood CT have been reported (13-16). In our study, the frequency of CT (21.7%) was appeared to be higher than that previously reported by a multicenter study summarizing the data of the Turkish Society of Pediatric Hematology (8.9%) (14). The reason for this difference may be that our center is one of the largest centers for the treatment of CHD in our country. Our demographic data revealed that 65.8% of patients with CT, all patients with RV thrombosis, and half of the patients with LA thrombosis were older than one year. Two studies from Turkey reported findings similar to ours. In one of them all 13 patients who

presented with intracardiac thrombosis were older than one year (15). The other study revealed that 13 of 14 patients with CT were older than one year (16). In our study, 11 of 22 patients with CT and CHD were also older than one year, perhaps because most patients who undergo cardiac surgery at our center are older than one year.

Our study, like similar studies published in the literature, showed that all patients with CT had an underlying associated clinical condition, mostly CHD (12,17,18). Our data strongly points out that congenital cardiac malformations were the major cardiac disorder that causes the development of CT in children. Although a formal comparison was not performed, having cyanotic or acyanotic CHD seems to made no difference in the incidence of thrombosis in our patients. In literature, there is no clear evidence that links cyanotic or acyanotic CHD with CT, perhaps because few studies on that topic have been published (16). Our study disclosed that the number of CT patients with cyanotic and acyanotic CHD were nearly equal. Since hemoglobin level and blood viscosity are high in patients with cyanotic CHDs, a tendency to thrombosis is expected (17). We believe a larger sample of subjects in a multicenter study may ensure reliable results.

Dilated cardiomyopathy was one of two major risk factors for CT together with CHD in above-mentioned study by Gurgey and colleagues (16). In that study, the prevalence of CT in patients with cardiomyopathy was 8.5%. A study by Tegeler and Downes showed that the frequency of CT in patients with dilated cardiomyopathy was 71% (4). In our study, incidence of CT was similar to the study by Gurgey et al. The significant difference between our results and Gurgey and colleagues study compared to the research by Tegeler and Downes further supports the need for a multicentre study (4,16).

Although a thrombus can form in any of the four cardiac chambers, the patients with CHD tend to have right heart thrombosis and those with cardiomyopathy have left heart thrombosis (4,16). Predisposing risk factors such as systemic infection, wearing a central venous catheter, or cardiac surgery are significant factors in the development of CT. In our study, wearing a central venous catheter was an important risk factor for the development of CT. In the literature, the incidence of thrombosis associated with central venous catheters ranges between 3.5-18.3% (9-30). In our study, the incidence of CT associated with central venous catheters (41.5%) was significantly higher than that previously reported (19-30). Although this finding might be the result of inadequate heparinization, retrospective analysis of our patients showed that was not the case.

Limited data have been reported in the literature about thrombophilic mutations in patients with CT because of the inadequate number of patients studied (15,16,24,31). In our study, 33 (80.5%) of 41 patients had at least one thrombophilic mutation and/or PAI-1 4G genotype. Furthermore, twenty-six patients (63.4%) had more than one thrombophilic mutation and/or PAI-1 4G allel. The prevalence of the FVL and Prt G20210A mutations in our study were higher than previously reported studies involving healthy Turkish population (32-36).

At least one MTHFR mutation was detected in 27/41 (65.8%) of our patients (heterozygote and/or homozygote) (Table 3). We also detected hyperhomocysteinemia (53.7%) rate similar to MTHFR mutation ratio. These findings are similar to the information in literature (10,37,38).

In conclusion, our data strongly suggest that underlying disorders such as CHD, malignancy, and cardiomyopathy and clinical risk factors such as cardiac surgery, central venous catheters, and systemic infections are important contributors for the development of CT. The type of disorder usually determines the site of thrombosis. This study also shows that to ensure early diagnosis, routine screening for CT should be performed in patients who have undergone an invasive procedure. Our results strongly suggest that the FVL and Prt G20210A mutations can be predisposing molecular risk factors for CT in these patients. We believe that screening for the

FVL mutation in countries with high prevalence should be included in the examination of all patients with a CT, whether or not a predisposing factor was identified. More aggressive prophylaxis may be suggested in such a situation.

List of abbrevations

ACA: anti-cardiolipin antibodies
CGVT: cardiac and/or great vessel thrombosis
CHD: congenital heart disease
CT: cardiac thrombosis
FVL: factor V Leiden
Ig: immunoglobulin
lp(a): lipoprotein (a)
MTHFR: methylene tetrahydrofolate reductase
PAI: plasminogen activator inhibitor
PCR: polymerase chain reaction
Prt: prothrombin

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Namik Yasar Ozbek, Fatma Belgin Atac and Baris Malbora planned and performed experiments, and wrote the manuscript. Zekai Avci, Hasibe Verdi, Bulent Alioglu, and Birgül Varan performed experiments. There is no potential conflict of interest to disclose.

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