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ARAŞTIRMA/RESEARCH

Retrospective evaluation of pediatric inpatients admitted with thrombosis according to risk factors: single center experience

Trombozla başvuran pediatrik hastaların risk faktörleri açısından retrospektif olarak değerlendirilmesi: tek merkez deneyimi

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Abstract

Purpose: Thrombosis in children is a multifactorial disorder associated with both genetic and acquired factors. Idiopathic thrombosis is a rare condition. This study aimed to examine the etiology and risk factors of thrombosis in pediatric patients followed at Baskent University Adana Teaching and Research Center, a reference hospital in the southern part of Turkey.

Material and Methods: This study included a sample of 49 pediatric patients who were retrospectively evaluated. The patient records were examined retrospectively in terms of age at diagnosis with thrombosis, gender, family history of thrombosis, localization of thrombosis, underlying primary disease and presence of catheter, as well as indicators of genetic risk factors.

Results: The number of children presenting to our clinic during the study period was 10358. The incidence of thrombosis was calculated as 49 cases in 10358 children. The median age of the patients was 5.1 years (ranging from 0 to 17.2 years), and there were 32 (65.3%) males. In the analysis of the genetic factors causing susceptibility to primary thrombosis, only a portion of the patients were studied for genetic mutations. Among these, 5 patients showed factor V Leiden mutation, 13 patients MTHFR 1298C mutation, and 18 patients MTHFR 677CT mutation. Seven patients were found have a combination of multiple mutations. When we investigated any possible correlation between high lipoprotein (a) levels and the distribution of mutations, we found that 7 patients with mutations had normal levels of high lipoprotein (a), while other 10 patients with mutations were found to have elevated levels of lipoprotein (a).

Conclusion: We conclude and strongly emphasize that lipoprotein (a) elevation is one of the important genetic risk factors, which might prove a major risk indicator, as valuable as other mutation screening tests performed by molecular genetics.

Key words: Thrombosis, risk factors, pediatric

Öz

Amaç: Çocuklarda tromboz hem genetik hem de kazanılmış faktörlerle ilişkilendirilmiş olup, multifaktöryeldir. İdiopatik tromboz nadirdir. Bu çalışmada, özellikle Türkiye'nin güney kesiminde önemli bir referans hastanesi konumunda olan Başkent Üniversitesi Adana Uygulama ve Araştırma Merkezi'nde tromboz nedeniyle izlenen pediatrik hastaların tromboz etyolojisi ve risk faktörlerinin gözden geçirilmesi planlanmıştır.

Gereç ve Yöntem: Tromboz nedeniyle izlenen 49 pediatrik hasta, retrospektif olarak değerlendirildi. Hastaların tromboz tanı yaşı, cinsiyeti, tromboz açısından aile öyküsü, trombozun lokalizasyonu, altta yatan primer hastalık ve kateter varlığı ile genetik risk faktörleri retrospektif olarak araştırıldı.

Bulgular: Tromboz tanısı konulduğu çalışma dönemindeki polikliniğimize başvuran çocuk sayısı 10358 idi. Tromboz insidansı 49/10358 olarak hesaplandı. Hastaların median yaşı 5,1 (0-17.2 yıl aralığında) olup, 32'si (%65.3) erkekti. Tromboza eğilim yaratan primer genetik faktörler araştırıldığında, genetik mutasyon analizi hastaların sadece bir kısmına yapılabildi. Bakılabilen hastaların 5'inde Faktör V Leiden mutasyonu, 13'ünde MTHFR 1298C mutasyonu, 18'inde MTHFR 677CT mutasyonu saptandı. Yedi hastada, birden fazla mutasyonun bir arada olduğu görüldü. Lipoprotein (a) yüksekliği ile mutasyon dağılımı arasında ilişki olup olmadığı araştırıldığında, mutasyon saptanan 7 hastada lipoprotein (a) düzeyi normal bulunurken, mutasyon saptanan 10 hastada yüksek lipoprotein (a) düzeyi bulundu.

Sonuç: Lipoprotein (a) yüksekliği, kalıtsal risk faktörlerinden biri olup, moleküler genetik yöntemleri ile taranan mutasyon tarama tetkikleri kadar önemli olabilecek bir risk faktörü olduğunu, yaptığımız çalışmada bulduğumuz sonuçla, önemle vurgulamaktayız.

Anahtar kelimeler: Tromboz, risk faktörleri, pediatric

INTRODUCTION

Thrombosis in children is a multifactorial disorder associated with both genetic and acquired factors. Idiopathic thrombosis is a rare condition, therefore, a child diagnosed with thrombosis should be examined for underlying risk factors, investigating any history of catheterization, infection, surgery, trauma, malignancy, autoimmune diseases and

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homocystinuria[1]. The genetic risk factors known to predispose to thrombosis often include somatic mutations causing activated protein C (APC) resistance, protein C deficiency, protein S deficiency and antithrombin III deficiency. A specific mutation, Factor V Leiden accounts for approximately 85% of the cases with APC resistance[2,3]. Patients with a known genetic risk factor always carry the risk of developing thrombosis triggered by an acquired stimulus.

This study aimed to examine the etiology and risk factors of thrombosis in pediatric patients followed at Baskent University Adana Education and Research Center, a reference hospital in the southern part of Turkey.

MATERIAL AND METHODS

Our study included a sample of 49 pediatric patients followed up for thrombosis at the Children's Clinic of Baskent University Adana Education and Research Center between December 2011 and August 2014, who were retrospectively evaluated. The patient records were examined retrospectively in terms of age at diagnosis with thrombosis, gender, family history of thrombosis, localization of thrombosis, underlying primary disease and presence of catheter, as well as indicators of genetic risk factors including APC resistance, mutations including Factor V Leiden, MTHFR 1298C, MTHFR 677, prothrombin G20210A, as well as the levels of protein C, protein S , antithrombin III, lipoprotein (a), activated partial thromboplastin time (aPTT), INR, fibrinogen, triglycerides, total cholesterol, vitamin B12, folic acid, homocysteine levels, anticardiolipin antibodies and lupus anticoagulant. The relevant data were transferred onto computer environment through a software package. The chi-square test was used for the intergroup comparisons in terms of frequency, mean and median values. As the variables failed to provide a parametric distribution assumption, the Mann-Whitney test was used to compare the chosen variables. Values of $p<0.05$ were considered statistically significant.

RESULTS

The median age of the patients was 5.1 years (ranging from 0 to 17.2 years), and there were 32 (65.3%) males. Four patients had a family history of thrombosis. 31 patients were diagnosed with central nervous system thrombosis, 6 deep vein thrombosis, 5 portal vein thrombosis, 2 deep vein thrombosis plus pulmonary embolism, 2 patients cardiac, deep vein thrombosis accompanied by pulmonary embolism, 2 extremity arterial thrombosis and 1 patient renal artery thrombosis.

Further investigation detected infections in 26 patients, catheter presence in 9 patients, surgical history in 5, malignant disease in 4, and gastroenterological disease in 4, cardiac disease in 4, renal disease in 2 patients, metabolic disease in 1 and traffic accident in 1 patient, and no underlying disease in 2 patients, who were considered to have primary thrombosis. The number of children presenting to our clinic during the study period was 10 358, include individual presentations. The incidence of thrombosis was calculated as 49 cases in 10 358 children

Recorded during thrombosis and evaluated retrospectively, the levels of aPTT, INR, fibrinogen, D-dimer (Figure 1), vitamin B12, folic acid, homocysteine (Figure 1), triglyceride, total cholesterol, and lipoprotein (a) (Figure 2), protein C, protein S, antithrombin III, lupus anticoagulant (Figure 4) were presented in charts. The parameters examined at the time of thrombosis showed high fibrinogen levels in the 50% of patients (n=12), low levels of protein C in 31.3% (n=10), high levels of triglyceride in 38.5% (n=10), low levels of protein S in 18% (n=6), lower folic acid values in 15.6% (n=5), high homocysteine in 17.4% (n=4), high total cholesterol in 14.4% (n=4), low vitamin B12 in 11.1% (n=3), and presence of lupus anticoagulant in 2 patients and a decrease in antithrombin III in 1 patient. In twenty-five patients, lipoprotein (a) levels could be measured, where 17 patients (68%) had lipoprotein (a) levels higher than the reference values.

In 5 of the 10 patients with low levels of protein C during thrombosis, the follow-up protein C levels were measured following the acute phase, where 4 of them still showed low levels. The low levels of protein S were detected in 6 patients, while only 2 of them were re-evaluated with follow-up measurements, which showed low levels of protein S in 1 patient. The antithrombin III levels were measured in 29 patients, and there was a significant decrease in only 1 patient, who was not re-evaluated after the acute period. None of the patients was

diagnosed with severe deficiencies of congenital protein C, protein S, and antithrombin III.

In the analysis of the genetic factors causing susceptibility to primary thrombosis, only a portion of the patients were studied for genetic mutations. Among these, 5 patients showed factor V Leiden mutation, 13 patients MTHFR 1298C mutation, and 18 patients MTHFR 677CT mutation. The heterozygous and homozygous distribution of these mutations were shown in Table-1. Seven patients were found have a combination of multiple mutations (Table-2). When we investigated any possible correlation between high lipoprotein (a) levels and the distribution of mutations, we found that 7 patients with mutations had normal levels of high lipoprotein (a), while other 10 patients with mutations were found to have elevated levels of lipoprotein (a). There was no statistically significant difference. During the follow-up period, 3 patients died of the primary disease and thrombosis-related complications.

Table-1. Distribution of detected mutations enhancing thrombosis in a group of patients.

	Factor V Leiden	MTHFR 1298C	MTHFR 677	Prothrombin G20210A
No detected mutations	28	11	12	26
Heterozygous mutations	4	9	14	-
Homozygous mutations	1	4	4	-
N/A	16 / 49	25 / 49	19 / 49	23 / 49

*N/A: Test not available

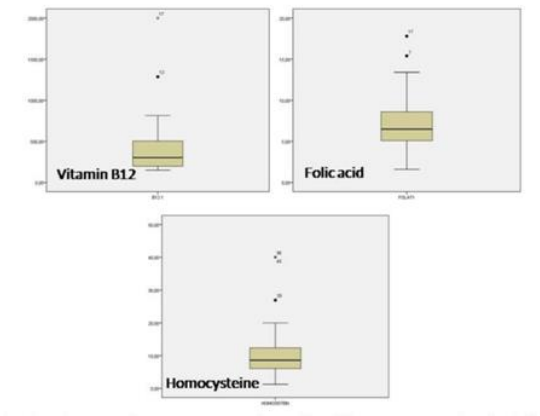

Figure 1. The distribution of Vitamin B12, Folic acid and homocysteine levels of the patients

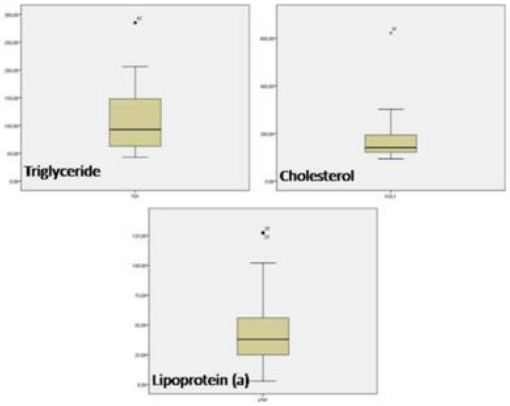

Figure 2. The distribution of triglyceride, cholesterol, lipoprotein (a) levels of the patients.

DISCUSSION

Although more prevalent in adult population with an incidence rate of 2.5 to 5%, thrombosis may also occur in pediatric population with an incidence of 0.07 cases per 10 000 children[4,5]. A study conducted by Gurgey et al reported that the incidence rate of thrombosis in children presenting to hospital was 78 cases per 100 000 children[6]. The incidence of thrombosis in our study was significantly higher (47.3/10 000) than their findings, whereas Ozbek et al found an even higher incidence of 88.6/10 000[7]. Even though thromboembolic events are less prevalent in children than in adults, they result in higher mortality and morbidity. Thrombosis is known as one of the most common complications occurring in children during treatment of the primary disease[4,5,8,9]. Recently, there has been an increase in the number of studies investigating thrombosis in children, both in Turkey and in the world[7,10-16].

Regarding the median age at the time of thrombosis diagnosis in children, Celkan et al reported a median age of 10 years, while Oren et al found this as 7 years, Tavil et al as 10.5 years[10,11,15]. The median age

of patients in our study was 5.1 years, which was significantly lower than the findings of other studies. This may be associated with the onset of thrombosis recognized at an earlier age.

Higher frequency of catheter applications in newborn infants, endothelial damage as well as immature hemolytic system may be seen as the most important causes of thrombosis in this period[17,18]. Two of our 5 newborn infants in our study had a history of catheter implementation. We detected that one in every 2 infants diagnosed with thrombosis under the age of 1 years had a history of catheterization. None of our adolescent patients reported a history of catheterization.

Table-2. The patients carrying more than one thrombosis mutations.

Patient #	Factor V Leiden Heterozygous/Homozygous	MTHFR 1298C Heterozygous/Homozygous	MTHFR 677 Heterozygous/Homozygous
# 30	+/-	+/-	+/-
# 31	-/-	+/-	+/-
# 32	+/-	-/-	+/-
# 33	-/-	+/-	+/-
# 35	+/-	-/-	+/-
# 41	-/-	+/-	+/-
# 49	+/-	-/+	-/-

In our study sample, 4 patients (8%) had a positive family history of thrombosis. This finding is consistent with those of Ozyurek et al, who reported that 10 out of 113 (9%) pediatric patients with cerebral venous sinus (sinovenous) thrombosis had a family history of thrombosis[13]. In terms of the localization of thrombosis, 31 of our patients (63.3%) had thrombosis in the central nervous system. This localization was consistent with the results reported by Lawson, Celkan and Oren[10,11,19]. In the study sample of Ozbek et al, the most common localization was reported to be the intracardiac thrombus, which was explained by the fact that their host institution was a reference hospital for cardiovascular surgery[7]. Pulmonary thromboembolism is usually associated with deep vein thrombosis. In most cases, pulmonary thromboembolism follows a clinically silent course and may be masked by the symptoms of the underlying disease[8,15,20,21]. On the other hand, four of our patients (8.2%) had pulmonary thromboembolism. All cases of pulmonary embolism were accompanied by deep vein thrombosis and 2 cases by intracardiac thrombosis.

In our study sample, three patients (6%) had arterial thrombosis. Of these patients, one had renal artery thrombosis, while 2 had the extremity arterial thrombosis. Arterial thrombosis was reported to account for 26% of all pediatric thrombosis cases[22]. Although the incidence of arterial thrombosis in children has not been clearly identified in Turkey yet, Oren et al reported an incidence rate of 1.4%[11]. In patients with cardiac pathology, this rate was reported as 35.6% and 43.5%7,[14].

As for the predisposing factors, medical reasons, particularly infection and catheterization, are known to play a role in 76% of thrombosis cases detected in childhood[1,6,23-25]. In the study by Celkan et al, where catheterized patients and newborns with catheter-related thrombosis were excluded, the most common factors predisposing to thrombosis were found to be malignancy and infection[10]. In our study, however, approximately half of the cases were associated with infection, while catheterization ranked as the second most common factor. The fact that infection ranked first as a predisposing factor may be explained by the geographical location of the region with a subtropical climate, where the incidence of infection is significantly higher. In their study, Ozbek et al reported congenital heart disease as the most common predisposing factor in children with thrombosis, which was followed by infectious diseases. They explained this finding by the fact that the study environment was an important cardiovascular surgical center[7]. Although trauma is known to be one of the most important factors predisposing to thrombosis in adults, it is less common in children[25,26]. Ozyurek et al reported that only 3 in 113 pediatric patients with thrombosis had a history of trauma, while Ozbek et al found trauma history in 3 of 122 patients. Consistent with these results, we detected history of trauma in only 1 of our patients[7,13]. In our study, no underlying cause was found in 2 patients, who were considered to

have primary thrombosis. In the study by Ozbek et al, the number of cases defined as spontaneous thrombosis with no underlying causes was found to be 8 patients[7].

Table-3. Comparison of the mutations enhancing thrombosis with previous studies.

	Factor V Leiden (%)	MTHFR677 (%)	Prothrombin20210 (%)	Lipoprotein (a) (%)
Unal (2005)	21	-	2.7	10
Ozyurek(2007)	17.1	46.8	4.5	-
Alioglu (2008)	17.8	28.8	5.8	13.2
Tavil (2009)	12.5	6.25	-	18.75
Ozbek (2009)	14.1	40.7	4.4	19.3
Current study	15	60	-	68

In terms of the genetic risk factors for thrombosis, a Turkish study by Unal et al detected heterozygous Factor V Leiden in 8 of their 387 thrombotic patients (21%), heterozygous prothrombin G20210A mutation in 1 patient (2.7%)[12]. Ozyurek et al found that the most common genetic risk factor was MTHFR 677CT mutation with an incidence of 46.8%, followed by Factor V Leiden with 17.1%, and prothrombin 20210G mutation with 4.5%[13]. In a study by Alioglu et al, MTHFR 677CT mutation ranked first (28.8%), followed by FV Leiden (17.8%) and prothrombin 20210G mutations (5.8%)[14]. Tavil et al detected MTHFR 677CT mutation in 1 of the 16 patients diagnosed with pulmonary embolism (6.25%), FV Leiden mutation in 2 patients (12.5%)[15]. In a study by Ozbek et al, the MTHFR 677CT mutation was the most common with an incidence of 40.7%, respectively followed by FV Leiden mutation (14.1%) and prothrombin 20210GA mutation (4.4%)[7]. Broadly consistent with earlier research, the most common mutation in our study was MTHFR 677CT (60%), and the second most common was MTHFR 1298C (54%), followed by FV Leiden mutation (15%) (Table-3). Our study also detected multiple mutations in 7 patients, while Ozbek et al reported the same in 6 patient, and Alioglu et al in 3 patients[7,14]. Contrary to the Western literature, publications from Turkey makes the impression that MTHFR is a common mutation in our society[7,13,14,27]. High levels of lipoprotein (a) is known as another hereditary risk factor in thrombotic patients. Lipoprotein (a) is a complex serum protein displaying similarities to plasminogen and inhibiting fibrinolysis, competing with plasminogen[28]. Serum levels higher than 30 mg/dL increase the risk of thromboembolism in childhood. In studies conducted in Turkey high levels of lipoprotein (a) were reported to range between 10% and 19.3%, whereas in our study, this ratio was significantly higher at 68% (Table-3). This may be particularly relevant to ethnic or regional; but no one studies in adult or pediatric patients related to this issue.

Thrombosis, although known to occur less commonly in children, is a serious condition where certain genetic risk factors should be taken into account. It is thought that these genetic risk factors vary across populations and geographic regions. Based on the findings of our study, we conclude and strongly emphasize that lipoprotein (a) elevation is one of the important genetic risk factors, which might prove a major risk indicator, as valuable as other mutation screening tests performed by molecular genetics. Further research on this topic is therefore recommended.

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