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Methyltetrahydrofolate Reductase C677T Mutation and 4G/5G Plasminogen Activator Inhibitor-1 Polymorphism in a Child with Deep Vein Thrombosis

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Introduction

Methyltetrahydrofolate reductase (MTHFR) enzyme is responsible for conversion of 5, 10-methyl tetrahydrofolate to 5-tetrahydrofolate for folic acid (folate) synthesis. One of the most common MTHFR mutations is a nucleotide transition from C-to-T at position 677 that results in amino acid alteration from alanine to valine. Coinheritance of MTHFR mutation with Plasminogen activator inhibitor-1 (PAI-1) polymorphism is a rare event which could increase the risk of coagulation and thrombosis.

In this manuscript, we describe a 2.5- year- old Iranian girl who was admitted with deep vein thrombosis (DVT) and suffered from both disorders.

Case Report

An Iranian 2.5-year-old girl was admitted to Mofid Children Hospital with chief complaint of right lower extremity pain for 5 days. She suffered from right upper and lower extremity muscular weakness since birth. She had normal cognitive development. She was born prematurely at 36 weeks gestational age through cesarean section with birth weight of 1990 gr. The positive findings on her physical examination were discrepancy between circumference of lower extremities (right mid calf = 22 cm, right mid thigh= 26 cm, left mid calf = 20 cm and left mid thigh= 22 cm), and decreased range of motions in both right hip and knee joints. Her initial white blood cell count was 16300/mm^3 with 50% polymorphonuclear (PMN) and 50% lymphocytes. Red blood cell count was 5180000/mm^3, hemoglobin value 12.9gr/dl, mean corpuscular volume (MCV) 73.9fL, mean corpuscular hemoglobin (MCH) 24.9pg, mean corpuscular hemoglobin concentration (MCHC) 33.9g/dl, platelet count 250000/mm^3, and ESR 40 mm/hr. Results of coagulation tests are shown in table 1.

Right lower extremity ultrasonography study revealed hyperechoic areas in right femoral vein and soft tissue inflammation without hip joint effusion. In Doppler ultrasonography femoral vein thrombosis was confirmed. We administered heparin (20U/Kg/hr) and controlled partial thromboplastin time (PTT). After 5 days, we added warfarin to the treatment regimen. Her signs and symptoms were relieved and warfarin was continued. She had no problem in follow up 7 months after the first admission.

She was admitted again after 7 months with pain, limping, and inflation of left lower extremity. On physical examination, the circumferences of left mid calf and mid thigh were 3cm, and 5cm larger than right mid calf and mid thigh, respectively. In Doppler ultrasonography, there was a large thrombosis in common femoral vein and left popliteal vein. Laboratory tests revealed that white blood cell count was 9100/mm^3 with 62% PMN and

***Table 1**. Results of coagulation tests on the first admission.*

Test	Result	Units	Reference Range
Platelet Count	250000	/mm³	150000-450000
A PTT patient	28	Sec	25-33.5
A PTT control	30.5	Sec	25-33.5
PT patient	13	Sec	12.6-15
PT control	13.8	Sec	12.6-15
INR	1.1	-	1-1.3
BT (Ivy method)	3	min	3-7
Protein C activity (3 times were checked)	57 88 95	%	70-130
Protein S activity	115	%	55-123
Antithrombin III	104	%	80- 120
Anti-phospholipid Ab(IgG)	6	U/ml	<11
Anti-phospholipid Ab(IgM)	4	U/ml	<11
Homocysteine	7	µ mol/L	5-15
Factor II activity (1-stage method)	76	%	50-150
Factor V activity	94	%	50-150
Factor VII activity	54	%	50-150
Factor VIII activity	142	%	50-150
Factor IX activity	78	%	50-150
Factor X activity	86	%	50-150
Factor XI activity	114	%	50-150
Factor XII activity	69	%	50-150
Factor XIII Screen	Normal	min	>30
Factor V Leiden PCR	Wild type Homozygous-Normal		
G20210A mutation PCR	Wild type Homozygous-Normal		

32% lymphocytes. Hemoglobin value was 11.6gr/dl, and platelet count 201000/mm³. The results of coagulation tests are mentioned in table 2.

Her treatment was started with Enoxaparin (1mg/Kg/dose, bidaily, subcutaneously) for 7 days, and continued with Warfarin until the results of laboratory evaluations were prepared. Since then she takes folic acid (5mg daily, orally), Vitamin B6 (1mg daily, orally), vitamin B12 (1mg monthly, intramuscular injection), and aspirin (80mg daily, orally) in her follow up. There has not been any signs of thrombosis recurrence in the last 6 months.

Discussion

Venous thrombosis is the result of the combination of various genetic and environmental factors. MTHFR is the enzyme that catalyses the transformation of homocysteine to methionine via the remethylation pathway (gene located in 1p36). Hyperhomocysteinemia (HHC), a known prothrombotic condition, is the consequence of decreased activity of MTHFR. Interestingly, both genetic and acquired factors may lower the activity of MTHFR. The C677T mutation, causing an amino acid change from alanine to valine, making the enzyme thermolabile, and halving its efficiency, is the most common genetic cause of HHC.[1] However this mutation is associated with elevated homocysteine levels only in the case of low folate intake.[2]

Conflicting results have been reported regarding the prothrombotic role of the C677T variant, which in fact seems far less pronounced than that of FV Leiden or prothrombin 20210A.[3,4] The prevalence varies widely in different populations (allelic frequency: 0.06–0.59; homozygosity frequency:(0–0.35),[5] and seems closely related to folic acid content of food.[2,6] In Europe (and also in Asia), there is a North to South gradient with a very high prevalence among Mediterranean countries.[7]

Plasminogen activator inhibitor-1 (PAI-1) disease is a relatively less common genetic defect caused by

Table 2. *The results of coagulation tests on the second admission.*

Test	Result	Units	Reference Range
Platelet Count	241000	/mm³	150000-450000
A PTT patient	28	Sec	28-38
A PTT control	33	Sec	28-38
PT patient	10.5	Sec	10-13
PT control	10	Sec	10-13
INR	1.05	-	1-1.3
BT (Ivy method)	3	min	3-7
Fibrinogen(Clauss method)	2.57	g/L	1.5-4.5
TT	18	sec	17-27
Protein C activity	93	%	70-130
Protein S activity	84	%	58-140
Antithrombin III	106	%	80- 120
Lupus anticoagulant	2	Sec	<8 Negative
Anti-phospholipid Ab(IgG)	2.5	U/ml	<10 Negative
Anti-phospholipid Ab(IgM)	2.3	U/ml	<10 Negative
Anti-cardiolipin IgG	1.8	GPL u/ml	<15
C3	226	mg%	90-180
C4	43	mg%	10-40
CH50	144	U	70-150
ANA	4.4	U/ml	<10
Anti-ds DNA	27	IU/ml	<40
Anti-PR3(cANCA)	0.1	U/ml	<0.4
Homocysteine	7	µ mol/L	5-15
MTHFR PCR		Mutant Homozygous	
PAI-1 polymorphism PCR		Mutant Homozygous	

defects in the PAI-1 gene transcription promoter region. It is a 4G/5G type polymorphism in the promoter region. Certain genotypes (4G/4G and 4G/5G) will be associated with increased plasma levels of PAI-1 protein. PAI-1 enzyme inactivates tissue plasminogen activator (tPA) enzyme, which is responsible for the activation of plasminogen to plasmin that digests the fibrin/blood clots. Thus, elevated levels of PAI-1 will lead to a hypofibrinolytic and hyperthrombotic status.

The coinheritance of these relatively common genetic conditions, which is not a rare event, further increases the relative risk of thrombosis. Some of the previously reported conditions include FV Leiden plus prothrombin 20210A,[8,9] or either of them plus HHC with or without the proven presence of the C677T MTHFR variant.[9-11] Similarly, the combination with an environmental risk factor is associated with a substantial increased risk of venous thromboembolism.[12,13]

Coinheritance of MTHFR mutation with PAI-1 polymorphism is a rare event which could increases the risk of thrombosis. Fujimura et al reported that MTHFR mutation increases the risk of thrombosis especially in homozygote patients (VV genotype).[14] Individuals with VV genotype showed higher plasma homocysteine (15.4±6.9nmol/ml) than did heterozygotes with AV genotype (11.2±3.7nmol/ml, p=0.009) or normal individuals with AA genotype (11.1±4.7nmol/ml, p=0.004).[14] Thus, mean homocysteine levels in plasma of homozygotes were significantly higher than in heterozygotes or normal individuals.[1,15] One report identified relatively low prevalence of the VV genotype in the control group (4%) and moderate prevalence of the VV genotype in patients with DVT (11%). They reported the VV genotype as a genetic risk factor for DVT.[2]

Despite coinheritance of MTHFR mutation with PAI-1 polymorphism in our patient, homocysteine levels in both admissions were normal. However, false negative and normal homocysteine level has been reported in MTHFR mutation and rechecking is advised.[16] It shows the necessity of checking for

MTHFR mutation in the case of hypercoagulability status, even with normal serum homocysteine. It may be related to high folate diets or increased activity of bypassing enzyme pathways. On the other hand, coinheritance of PAI-1 mutation in our patient explains the repeated thrombotic event, despite normal homocysteine level.

It is noteworthy to take in mind the possibility and importance of coinheritance of even rare genetic disorders which could lead to thrombotic events especially in younger children.

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