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Hyperhomocysteinemia and C677T MTHFR Genotype in Patients With Retinal Vein Thrombosis

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Abstract

Introduction: Elevated homocysteine (Hcy) is associated with the risk of deep vein thrombosis, pulmonary embolism, ischemic heart disease, and stroke. Several studies have suggested that hyperhomocysteinemia (HHcy) may predispose to retinal vein thrombosis (RVT) development. The aim of this study is to investigate the relationship between Hcy, C677T methylenetetrahydrofolate reductase (MTHFR) genotype, and RVT in patients compared with controls.

Materials and Methods: We evaluated the Hcy plasma level of 3114 consecutive participants in 2 Italian centers during a 2-year period. Hyperhomocysteinemia was found in 99 patients and 136 healthy participants. Of the 99 patients, 20 had RVT with a high prevalence of HHcy in the RVT subgroup (20.2%). This result suggested a possible relationship between HHcy and RVT development. We investigated 105 consecutive patients with recent diagnosis of RVT, and we compared them with 226 healthy controls to evaluate whether HHcy may be a risk factor for RVT.

Results: the prevalence of HHcy was higher in patients compared with controls (34.3% vs 14.2%; $P < .001$). The MTHFR C677T genotype was found in 69 of 105 (65.7%) patients with RVT (heterozygosity: 40 of 105 and homozygosity: 29 of 105). The control group showed the presence of MTHFR C677T genotype in 169 of 226 participants (74.8%; heterozygosity: 100 of 226 and homozygosity: 69 of 226) without difference between the 2 groups ($P = .08$).

Conclusion: our study suggests that HHcy is a possible risk factor for RVT development, while no association was found between RVT and the C677T MTHFR genotype.

Keywords

thrombophilia, venous thromboembolism, gene polymorphisms

Introduction

Retinal vein thrombosis (RVT) is an important cause of visual impairment and blindness, especially in the elderly individuals. It is considered a multifactorial disease that may affect small, medium, and large ocular vessels; central occlusion represents the most dangerous clinical entity. However, RVT is considered an unusual site of thrombosis.[1] In this study, we analyzed the clinical data from patients evaluated for thrombophilia. Previous studies have shown an association between RVT and hypertension, diabetes mellitus, dyslipidemia, genetic prothrombotic risk factors (factor V leiden [FVL] and prothrombin [PT] G20210A), deficiencies of physiological coagulation inhibitors (antithrombin and proteins C and S), and presence of antiphospholipid antibodies.[2]

Several collagen-vascular diseases (systemic lupus erythematosus, temporal arteritis, scleroderma, etc.) may also cause RVT. Patients with malignant diseases are at substantial risk of thromboembolism from tumor-induced hemostatic abnormalities leading to a hypercoagulable state.[3] Patients with chronic kidney disease (CKD) have a much higher risk of cardiovascular disease and mortality than the general population.[4]

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Data from earlier case-control studies suggested that hyperhomocysteinemia (HHcy) may be a risk factor in RVT.[5-9] However, not all studies demonstrated that plasma HHcy is related to RVT.[10-12] The aim of this study was to analyze the relationship between plasma homocysteine (Hcy) levels, C677T methylenetetrahydrofolate reductase (MTHFR) genotype, and RVT to evaluate whether HHcy may be a risk factor for RVT development in a group of patients compared with healthy controls.

Homocysteine is an amino acid formed from methionine and its metabolism is dependent on vitamins or vitamin derivatives (folate, vitamin B12, vitamin B6 and flavin adenin dinucleotide). It is generally accepted that a mildly elevated Hcy level is a risk factor for vascular diseases.[13] Increase in HHcy may trigger endothelial dysfunction through oxidative damage therefore inducing increased oxidation of low-density lipoprotein and hypercoagulable state. The toxic effect of high Hcy concentrations on endothelial cells also results in an increased platelet adhesion, impaired regulation of endothelium-derived relaxing factor, induction of tissue factor, suppression of heparan sulfate expression, and stimulation of smooth muscle cell proliferation.[14] Several mechanisms may lead to HHcy. Predisposing factors include different diseases (renal failure, hypothyroidism, diabetes type II, proliferative diseases, etc.); drugs (metformin, theophylline, methotrexate, anticonvulsivant, omeprazole, etc.); deficiencies of folate, vitamin B6, and vitamin B12. An important cause of elevated plasma Hcy level is the C677T polymorphism in the MTHFR gene that is common in Western population. Homozygous participants for the C677T MTHFR show higher plasma levels of Hcy, particularly associated with low serum folate levels.[15] HHcy is a well-known risk factor for thrombotic disease but actually is unclear whether elevated plasma concentrations of Hcy are also associated with retinal vascular disease.[16]

Material and Methods

The first step of this study was a revision of the clinical data from a cohort of 3114 consecutive participants visited in the Italian Thrombosis Centers of Reggio Calabria and Catanzaro in a 2-year period (from June 2006 to June 2008) for a thrombophilic evaluation. From the evidence of a high percentage of patients with RVT in the subgroup with HHcy, we hypothesized a relationship between RVT and HHcy as independent risk factor of thrombosis.

In the second part of the study we investigated 105 patients (46 males and 59 females; aged 22-86 years, mean 58.4) with RVT (88 cases with central retinal vein occlusion [CRVO] and 17 cases of branch retinal vein occlusion), from June 2006 to June 2008. We included only cases of RVT diagnosed within 3 months before the enrollment.

Patients were compared with 226 healthy participants (129 males and 97 females; aged 18-86 years, mean 55.7) without a history or clinical evidence of retinal vascular diseases, recruited during the same 2-year period.

The healthy controls were relatives of patients with a history of thromboembolism or women who underwent screening for thrombophilia before taking oral contraceptives.

Patients and controls underwent laboratory evaluation for fasting total Hcy plasma; physiological clotting inhibitors (proteins C and S and antithrombin); presence of activated protein C resistance (aPCR), antiphospholipid, and anticardiolipin antibodies. The G20210 PT gene (factor II [FII] G20210A), FVL, MTHFR C677T polymorphism, and folate and vitamin B12 levels were also evaluated. Exclusion criteria were the evidence of renal failure (3 patients), genetic prothrombotic risk factors (8 patients with FII G2010A and 7 with FVL mutations), antithrombin deficiency (1 patient), presence of antiphospholipid antibodies (1 patient), other vascular diseases (4 patients), and cancer (3 patients).

All patients and controls gave written informed consent. The study protocol was approved by the Ethical Committee of the Riuniti Hospital of Reggio Calabria.

Laboratory Testing

Plasma samples. Whole blood samples were collected by venipuncture from antecubital vein. Samples for coagulation assays were collected into vacutainer tubes containing 1/10 volume of 0.105 M trisodium citrate; platelet-poor plasma was obtained by centrifugation at 4000*g* for 8 minutes and then stored frozen at –70°C until used.

Antithrombin. Automated determination of antithrombin activity in the plasma was performed using an automated chromogenic assay in human citrated plasma on IL Coagulation Systems (Instrumentation Laboratory, Italy).

Free Protein S. The evaluation of free protein S was performed with the automated latex ligand immunoassay for the quantitative determination of free protein S (IL Coagulation Systems, Instrumentation Laboratory).

Protein C. The determination of protein C was performed using an automated chromogenic assay (IL Coagulation Systems, Instrumentation Laboratory).

Lupus Anticoagulant. Screening assays included the kaolin clotting time, dilute activated partial thromboplastin time (APTT), dilute PT, and 2 dilute Russell viper venom times; all tests were repeated in a 1:1 mix with normal plasma, and positive test results were confirmed with the same reagent in the presence of excess phospholipids (IL LAC Kit, Instrumentation Laboratory).

Anticardiolipin antibodies. Detection, characterization of specificity, and determination of the immunoglobulin G (IgG) or IgM isotype of Anticardiolipin antibodies (aCL) were performed by enzyme-linked immunosorbent assay (ELISA), using commercially available kits (Anti-Cardiolipin, GA Generic Assays, Germany)

Table 1. Characteristics of Retinal Vein Thrombosis (RVT) in Patients and Controls

	RVT patients		Controls		
	No	%	No	%	*P* value[a]
Total	105	100	226	100	
Gender					
Male	46	44	44	19	<.001
Female	59	56	182	81	
Age (years)					
<30	17	16	175	77	<.001
30-34	21	20	31	14	
35-39	29	28	11	5	
>40	38	36	9	4	
C677T MTHFR genotype					
Absent	36	34	57	25	.23
Heterozygosity	40	38	99	44	
Homozygosity	29	28	70	31	
Homocysteine level (μmol/L)					
<16	69	66	194	86	<.001
16-20	12	11	15	7	
21-30	16	15	12	5	
>31	8	8	5	2	

[a] Chi-square test.

Homocysteine. The determination of Hcy was performed by enzyme immunoassay (EIA) test (Microplate Enzyme Immunoassay, Bio-Rad Laboratories Italy). An elevated Hcy level was defined as greater than 15 μmol/L.

Factor V Leiden, PT, and MTHFR mutations. The investigation for the FVL, FII G20210A, and MTHFR C677T gene polymorphisms was made by venous blood samples collected into EDTA containing vacutainer tubes; the assays were based on the reverse hybridization principle, including 3 successive steps: isolation of DNA from peripheral blood, in vitro amplification of gene sequences, and hybridization of the amplification products with allele-specific oligonucleotides probes (Vienna Lab, Labordiagnostika GmbH).

Statistical Analysis

The distribution of HHcy (categorized in 4 levels: <16, 16-20, 21-30, >31 μmol/L) and MTHFR C677T polymorphism among RVT patients and controls was tested using chi-square (χ^2). We evaluated the risk of RVT using Hcy level and C677T MTHFR by calculating the odds ratios (ORs) and 95% confidence intervals (95% CI) using multiple logistic regression; we included the following covariates in the model: gender, age class (<45, 45-54, 55-64, >65 years). Statistical analyses were performed using the software Stata, Version 10[17].

Results

The first revision of the clinical data from a cohort of 3114 participants investigated for thrombophilia showed 235 (7.5%) participants with HHcy (Hcy level higher than 15 μmol/L). Of the 235, 138 were men and 97 were women; the median age was 55.7 years (range: 18-86). Of them, 99 had a thromboembolic event: 17 deep venous thrombosis, 9 pulmonary embolism, 14 myocardial infarction, 31 stroke, 4 arterial thrombosis, 4 thrombophlebitis, and 20 RVT. The remaining 136 were healthy participants; the total number of healthy participant studied were 958. We found a large number of patients with RVT and HHcy (20.2% of all thrombotic events) if compared to the number of thrombosis in more common sites. This evidence leads us to evaluate a possible relation between RVT and HHcy. We investigated 105 consecutive patients with a recent diagnosis of RVT and 226 healthy controls (Table 1). In both the groups, the folate and vitamin B12 levels were within the normal range. The control group was mainly composed of women. Distribution of C677T MTHFR genotype was similar among the 2 groups, while that of HHcy (Hcy >15 μmol/L) was markedly different: HHcy was found in one third of patients with RVT (Hcy levels ranged from 16 to 61 μmol/L; average 27.3) and in 14% of controls (range: 16 to 52 μmol/L; average 23.7). In control group, we found 35 participants with FVL or FII G20210A (1 homozygous for FVL, 16 heterozygous for FVL, 1 homozygous for FII G20210A, 16 heterozygous for FII G20210A, 1 heterozygous for both FVL and FII G20210A).

Prevalence of HHcy was higher among participants with C677T MTHFR homozygosity in both patients (52% vs 22%-33% in heterozygous-absent genotype) and controls (27% vs 11%-4%; Table 2). Antithrombin and proteins C and S levels were normal in patients. We did not find FII G20210A or FVL polymorphisms, aPCR, lupus anticoagulant, or aCL antibodies in any patients enrolled.

In a multiple logistic regression model adjusted for gender and age, we confirmed the positive association between RVT risk and Hcy levels observed in the univariate analysis (*P* value for trend = .001): in particular, the risk was increased 5 to 6 times among with participants with Hcy level above 20 and 30 μmol/L, respectively (Table 3); no increased risk was found between 16 and 20 μmol/L.

In a gender and age-adjusted multiple regression model, we also confirmed the lack of association of C677T MTHFR genotype with RVT risk: compared to participants without the genotype, the OR was 0.86 for heterozygous participants and 1.01 for homozygous participants.

Discussion

Information about the pathogenesis of retinal vascular diseases is still poor and little data are available in literature: coagulation abnormalities, changes in blood vessel walls, and systemic conditions such as hypertension, atherosclerosis, and diabetes mellitus seem to be possible risk factors associated with RVT development. However, in many cases none of these factors are present, and the question of hidden risk factors is raised.[18] In the past years, several scientific reports have suggested that HHcy may be related to an increased risk of retinal occlusion

Table 2. Prevalence of Hyperhomocysteinemia (HHcy) and C677T Methylenetetrahydrofolate Reductase (MTHFR) Genotype in Patients and Controls

	C677T MTHFR genotype			
HHcy	Absent	Heterozygosity	Homozygosity	Total
Patients				
Absent (<16 μmol/L)	24, 67%	31, 78%	14, 48%	69, 66%
16-20 μmol/L	7, 19%	2, 5%	3, 11%	12, 11%
21-30 μmol/L	5, 14%	4, 10%	7, 24%	16, 15%
>31 μmol/L	0, 0%	3, 7%	5, 17%	8, 8%
Total	36, 100%	40, 100%	29, 100%	105, 100%
P value (chi-square test) = .03				
Controls				
Absent (<16 μmol/L)	55, 96%	88, 89%	51, 73%	194, 86%
16-20 μmol/L	1, 2%	7, 7%	7, 10%	15, 7%
21-30 μmol/L	0, 0%	4, 4%	8, 11%	12, 5%
>31 μmol/L	1, 2%	0, 0%	4, 6%	5, 2%
Total	57, 100%	99, 100%	99, 100%	226, 100%
P value (chi-square test) = .003				

Table 3. Risk of Retinal Vein Thrombosis (RVT) by Selected Variables

Variable	OR	95% CI	*P* value
Gender (M vs F)	1.59	0.78-3.27	.20
Age class (vs <45 years)			
45-54	7.03	3.09-15.99	<.001
55-64	33.70	13.46-84.36	<.001
>65	46.02	17.61-120.22	<.001
Homocysteine (vs <16 μmol/L)			
16-20	1.04	0.33-3.26	.94
21-30	4.80	1.58-14.63	.006
>31	6.06	1.43-25.75	.015
C677T MTHFR genotype (vs absent)			
Heterozygosity	0.86	0.40-1.84	.70
Homozygosity	1.01	0.45-2.25	.99

OR = odds ratios calculated with multiple logistic regression model containing all the indicated variables; 95% CI = 95% confidence interval.

but not all authors confirmed a possibility of an active role of MTHFR C677T genotypes in the retinal vascular occlusions.

Cahill[6] found that mean HHcy level was higher in 87 Irish patients with retinal vascular occlusive disease (RVOD) but the MTHFR C677T homozygosis was not associated with RVT. Weger[19] also supported that HHcy, but not the MTHFR C677T mutation, is associated with CRVO. Terrazzi and colleagues[1] suggested that HHcy can be considered a risk factor for retinal vein occlusion, and observed that MTHFR C677T homozygosis is more frequently associated with retinal vein occlusions. In addition, Marcucci et al[9] showed that the homozygosity for C677T MTHFR polymorphism was associated with higher Hcy plasma levels in a group of 100 consecutive patients with CRVO.

In the present retrospective case-control study, we investigated the role of HHcy and MTHFR C677T gene polymorphism on RVT development. The results of our investigation support the hypothesis that Hcy plasma level can be considered a risk factor for RVT. One of the possible causes of HHcy is the presence of C677T MTHFR polymorphism. In our study, we found a difference in Hcy levels but the prevalence of C677T MTHFR did not differ between patient and controls; this could be explained with the high prevalence of C677T MTHFR in Italians.[20,21]

At the same time, a possible evidence of the role of C677T MTHFR homozygosity in increasing Hcy levels in patients with RVT seems to be confirmed by the high prevalence of C677T MTHFR homozygosis and HHcy in patients withRVT.

Our study did not evidence a clear association between C677T MTHFR and RVT. If the assessment of Hcy is important in the investigation, in the management, and in the follow-up of patients with RVT, C677T MTHFR genotype cannot be considered a risk factor for RVT, so there is no rationale for measuring this variant for clinical purposes.

Homocysteine-decreasing treatment with vitamins (folic acid, pyridoxine, and cyanocobalamin) seems to reduce cardiovascular risk in patients with HHcy correlated with genetic defects of Hcy metabolism.[22,23] In this study, we did not investigate this important aspect, therefore an appropriate clinical trial should clarify whether folic acid and B vitamins that decrease the risk of RVT is needed; it could be as much important that further prospective studies could confirm the benefits ratio of lowering Hcy level in patients with a first episode of RVT for the prevention of recurrence of retinal vascular occlusions.

Declaration of Conflicting Interest

The authors certify that they have no affiliation with or financial involvement in any organization or entity with a direct financial interest in the subject matter or materials discussed in the manuscript.

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