Buerger's Disease and Hyperhomocysteinemia: Is there a Relationship?

Pierpaolo Di Micco[*,1], Rosanna Di Fiore[2,3], Gianluca Di Micco[1], Giuseppe Cardillo[2,3], Chiara Bellia[4], Sandro Quaranta[2,3], Marcello Ciaccio[4] and Giuseppe Castaldo[2,3]

[1]*Buonconsiglio Fatebenefratelli Hospital of Naples, Naples, Italy*

[2]*Dipartimento di Biochimica e Biotecnologie Mediche, Università di Napoli Federico II, Naples, Italy*

[3]*CEINGE Biotecnologie Avanzate, Scarl, Naples, Italy*

[4]*Cattedra di Biochimica Clinica, Università di Palermo, Italy*

Abstract: Thromboangiitis obliterans, also known as Buerger's disease, is a cause of juvenile lower limb ischaemia. Buerger's disease is idiopathic and one of diagnostic criteria is the absence of atherosclerotic risk factors other than smoking. A possible involvement of thrombophilia has been investigated and the role of hyperhomocysteinemia is still matter of discussion. We describe 9 patients with Buerger's disease followed-up for the past 3 years. We found a significant increase in circulating homocysteine levels (mean: 31.6 in patients *vs* 8.2 μmol/L in control subjects). We also analyzed the C677T mutation of MTHFR; 5/9 Buerger's patients were heterozygotes and 4/9 homozygotes for the mutation as compared with 3 heterozygotes in the control group. Our data, although preliminary, suggest a possible role of homocysteine in the pathogenesis of Buerger's disease as one of the causes of endothelial dysfunction. The role of MTHFR C677T variant must be further evaluated in larger trials involving patients with Buerger's disease.

Keywords: Thrombophilia, hyperhomocysteinemia, MTHFR, Buerger's disease.

BACKGROUND

Lower limb ischaemia is usually due to atherosclerosis. In a small percentage of cases non-atherosclerotic diseases may determine reduced blood flow to the lower limb and/or arterial occlusion [1]. Among the non-atherosclerotic diseases are thrombophilic disorders such as cardiac or aortic embolism, antiphospholipid syndrome, inflammatory chronic diseases, systemic sclerosis or acute or chronic vasculitis, in particular Buerger's disease. Buerger's disease is a thromboangiitis obliterans of arterial or venous vessels of medium size due to an idiopathic inflammatory disease [2]. No clinical symptom, nor laboratory or instrumental signs (e.g. radiological, ultrasound or angiographic imaging) are specific for Buerger's disease and a probability approach has been proposed [2]. Mozes *et al.* in 1970 proposed diagnostic criteria and scores including early onset (i.e. < 40 years) and/or intermittent claudication and/or involvement of upper extremity vessels as the occurrence of migrating thrombophlebitis and/or Raynaud's phenomenon and/or radiological or histological features [3]. Similarly, Shionoya *et al.* suggested diagnostic criteria for Buerger's disease including early onset (i.e. < 40 years), history of smoking, intrapopliteal arterial occlusion, involvement of upper limb vessels, thrombophlebitis migrans and absence of further atherosclerotic risk factors other than smoking [4]. For this reason, patients with atherosclerotic risk factors other than smoking such as hypertension, dyslipidemia or diabetes usually did not show typical features of Buerger's disease. So, recently thombophilic conditions predisposing to a hypercoagulable state have been thought of as triggers for Buerger's disease [5].

The aim of this study was to search for a possible association between Buerger's disease and hyperhomocysteinemia in patients followed by our group.

PATIENTS AND METHODS

In the last 3 years, we selected 9 patients affected by Buerger's disease according to the diagnostic criteria described by Mozes and Shionoya [3, 4]. All patients underwent clinical observation for the onset of intermittent claudication with or without lower limb ischaemia. After examination by color-Doppler and angiographic evaluation all patients were assigned to treatment (pharmacological with or without surgical approaches).

We excluded patients with atherosclerotic risk factors other than smoking (i.e. age > 40 years, hypertension, diabetes or dyslipidemia) and patients with a hypercoagulable state due to inherited and/or acquired thrombophilia (i.e. factor V Leiden gene variant, presence of A202120G prothrombin variant, acquired activated protein C resistance, antiphospholipid syndrome, protein C deficiency, protein S deficiency, antithrombin deficiency), cardiac arrhythmia or chronic autoimmune disease (e.g. systemic erythematosus lupus or systemic sclerosis).

All selected patients gave their written informed consent and the study was approved by a local Ethics Committee.

All patients were tested for fasting homocysteinemia and for MTHFR C677T genotype using "PCR real time" (MTHFR C677T LCSet and the Light Cycler 1.2 Instrument -Roche Diagnostics-). We studied 9 age- and sex-matched

*Address correspondence to this author at the Internal Medicine and Emergency Room, Buonconsiglio Fatebenefratelli, Hospital of Naples, Naples, Italy; E-mail: pdimicco@libero.it

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healthy subjects as a control group without a history for recent vascular disorders (e.g. acute coronary syndrome and/or venous thromboembolism in the last year). No participants were on vitamin supplementation or antithrombotic drugs.

Serum creatinine levels were recorded to exclude an acquired cause of hyperhomocysteinemia. Creatinine values were within the reference range in patients and control subjects [1.05 ± 0.20 mg\dL (mean ± SD) in patients *vs* 0.95 ± 0. 22 mg\dL in control subjects].

STATISTICAL ANALYSIS

All statistics tests were performed using MatLab 7.6.0 R2008a (The Matworks Inc., Natick, MA, USA). Homocysteine serum level is a continue variable: the Anderson-Darling test for normality and the Levene's test for homoscedasticity were used to assess the opportunity to use the Student's t-test. The Randomisation test and the Fligner-Policello test, a robust, modified Mann-Whitney-Wilcoxon rank-order test for populations, which assumes neither normality nor equal variances, were performed to test differences between patients and controls in serum homocysteine levels.

The Hardy-Weinberg Proportion was assessed in both groups. Test on genetic data was performed using a Fisher's exact test for 2x3 matrix, when genotype was considered, or for 2x2 matrix, when alleles were taken in to account. Finally, odds ratio (OR) computation with Bayesian credibility assessment were performed to test association between alleles and Buerger's disease.

RESULTS

Table **1** summarizes the homocysteine values and MTHFR C677T gene variant of the Buerger's patients.

The Buerger's patients had homocysteine mean levels of 31.6 μmol/L *versus* 8.2 μmol/L for the controls. The Anderson-Darling test showed that homocysteine levels were normally distributed in the controls but not in the patients (Table **2**). Levene's test for variance equality showed heteroskedasticity (Table **2**). So, the parametric Student's t-test was not applicable. The best Box-Cox transformation for patients group was logarithmic: using this normalization procedure the Anderson-Darling test showed a normal distribution in both groups, but Levene's test was still positive (data not shown). So, 2 distribution-free tests were used and both showed that mean and median homocysteine serum levels in patients and controls were significantly different (Table **2**).

MTHFR C677T gene variant was present in all 9/9 patients compared with 3\9 of control subjects (Table **1**). Homozygosity for MTHFR C677T gene variant was present in 4/9 patients compared to none of control subjects; heterozygosity was present in 5/9 patients compared with 3\9 of control subjects. Both groups were in Hardy-Weinberg Proportion but in opposite direction caused by the different frequency of the T allele. Table **3** shows that genotypes and alleles distribution between patients and controls groups were significantly different. The odds ratio computed for the T allele is significant OR is 13 and the 95% confidence interval does not encompass 1 and credible (the OR is greater than critical value computed by Bayesian credibility assessment). Finally, the Φ parameter of association is 0.5031, showing that the T allele is a moderate risk factor.

DISCUSSION

Buerger's disease is a non-atherosclerotic occlusive disease characterised by thrombosis, inducing limb ischaemia and gangrene [2]. The disease is idiopathic and different from other immune arteritis, but there is a strong association with tobacco abuse [5]. On the other hand, hyperhomocysteinemia is a risk factor for both arterial and venous thrombosis, and also due to genetic defects such as MTHFR C677T gene variant and/or folate deficiency [6-9]. Tobacco abuse is also an important determinant of low folate levels [10]. In the past years, several association between Buerger's disease and alteration of haemostasis have been suggested [5]. In these reports a role of hyperhomocysteinemia playing a pathogenetic role in Buerger's disease was ruled out, but univocal data are lacking since the majority of reports were based on single case observations or small populations [5, 11, 12]. On the other hand, reports on the role of MTHFR C677T gene variant, a cause of hyperhomocysteinemia, as a trigger factor of Buerger's disease did not confirm this association [13, 14].

Table 1. Homocysteinemia and MTHFR C677T Gene Variant in the Patients with Buerger's Disease (Control Values are also Shown)

Patients	Homocysteine (μmol/L)	MTHFR C677T Variant	Control Subjects	Homocysteine (μmol/L)	MTHFR C677T Variant
M; age 29 years	65	Homozygous	M; age 30 years	9	Wild type
M age 25 years	19	Heterozygous	M; age 24 years	6.8	Wild type
M; age 31 years	34	Heterozygous	M; age 31 years	6.2	Wild type
M; age 37 years	15	Heterozygous	M; age 30 years	7.5	Heterozygous
M; age 38 years	71	Homozygous	M; age 42 years	10.4	Heterozygous
F; age 29 years	15	Homozygous	F; age 26 years	8.5	Wild type
M; age 38 years	25	Homozygous	M; age 39 years	9.4	Heterozygous
F; age 36 years	25	Heterozygous	F; age 38 years	8.3	Wild type
M; age 32 years	16	Heterozygous	M; age 33 years	7.9	Wild type
Mean	31.7		Mean	8.2	
SD	21.5		SD	1.3	
Median	25		Median	8.3	

M = male; F = female; SD = standard deviation.

Table 2. Statistical Tests Applied to Assess Differences Between Patients and Controls Groups Homocysteine Serum Levels

Applied Test	Homocysteine	
	Patients Group	Controls Group
Anderson-Darling test for normality (p-value)	0.0095	0.9928
Levene's test for homoscedasticity (p-value)	0.0015	
Randomisation test on means (1-tailed p-value)	0.0001	
Fligner-Policello test on medians (1-tailed p-value)	0.00002	

Table 3. Statistical Tests Performed to Assess Difference in Genotype and Alleles Distributions Between Patients and Controls Groups

MTHFR C677T Genotype	Patients Group	Controls Group
CC	0	6
CT	5	3
TT	4	0
Fisher's exact test (2-tailed p-value)	0.0047	
MTHFR C677T Alleles	**Patients Group**	**Controls Group**
C allele	5	15
T allele	13	3
Fisher's exact test (2-tailed p-value)	0.0020	
Power	0.9555	
Odds Ratio (OR) for T allele with 95% confidence interval	2.5919<13.0000<65.2037	
Bayesian Credibility Assessment: Critical Odds Ratio (COR)	3.6828 (OR>COR. Test is credible at the 95%)	
Φ	0.5031 Moderate positive association (risk factor)	

Our data showed hyperhomocysteinemia in Buerger's patients that was significant when compared with control subjects. We can speculate on a pathogenetic role of hyperhomocysteinemia in Buerger's disease but also a secondary increase in homocysteine levels because of the endothelial dysfunction already present in subjects with Buerger's disease cannot be excluded. On the other hand, the relationship with the MTHFR C677T gene variant reported here differs from previous published work [13, 14]. Further data are needed in larger populations with a wider ethnic distribution.

We suggest that the significant increase in homocysteine levels may have clinical implications if patients with Buerger's disease can benefit from vitamin supplementation (e.g. folic acid). This may provide a potential therapeutic option for these patients that frequently have a poor prognosis.

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