CLINICAL STUDY

Thrombophilia as a cause for central and branch retinal artery occlusion in patients without an apparent embolic source

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Abstract

Purpose **To assess the prevalence of vascular risk factors and thrombophilias in central and branch retinal artery occlusion in patients in whom an embolic source is not apparent.**
Methods **The study group consisted of 21 consecutive patients with retinal artery occlusion (RAO) in whom Doppler ultrasonography of the carotid arteries and transthoracic or transoesophageal echocardiography were normal. Laboratory methods included polymerase chain reaction for detection of factor V G1691A, factor II G20210A and methylentetrahydrofolate reductase C677T mutations, assays of plasma levels of protein C, free protein S, antithrombin, fibrinogen and homocysteine; and tests for the presence of lupus anticoagulant and anticardiolipin antibodies. Controls for the laboratory tests were 243 healthy subjects.**
Results **Nine of the 21 (43%) patients had at least one thrombophilic marker: 4 were homozygous for MTHFR C677T, 1 was heterozygous for factor V G1691A, 1 had a high titre of IgM anticardiolipin, 2 were heterozygous for factor V G1691A and homozygous for MTHFR C677T, and 1 had lupus anticoagulant, a high titre of IgM anticardiolipin, homozygosity for MTHFR C677T and hyperhomocysteinaemia. An interaction between vascular risk factors and thrombophilias seemed important since out of 14 patients with hypertension, diabetes and/or hypercholesterolaemia 7 (50%) had a thrombophilia. Homozygous MTHFR C677T was a significant risk factor with odds ratio of 3.18 (95% CI 1.20–8.47). The prevalence of factor V G1691A was also higher in the RAO patients versus controls with an odds ratio of 2.36 (95% CI 0.63–8.88), but this value did not reach significance, probably due to the small sample size.**
Conclusion **A search for thrombophilia in RAO is advisable in patients without evident source of emboli even when vascular risk factors are identified.**

Key words Retinal artery occlusion, Thrombophilia

Central retinal artery occlusion (CRAO) and branch retinal artery occlusion (BRAO), are clinical entities that profoundly compromise vision. CRAO and BRAO are frequently caused by thromboemboli or cholesterol emboli originating in atherosclerotic plaques of the carotid arteries, or by emboli originating in the heart, e.g. atrial fibrillation, aortic and mitral valve disease or prosthesis, subacute bacterial endocarditis and atrial myxoma.[1] It is therefore pertinent to search in all patients presenting with CRAO or BRAO for such sources of emboli by echocardiography (preferably transoesophageal) and Doppler ultrasound of the carotid arteries. In a significant number of patients, however, such imaging examinations fail to identify an embolic source[2] and hence a search for causes of *in situ* thrombosis due to arterial wall disease related to hypertension, diabetes mellitus, dyslipidaemia or vasculitis becomes indispensable. An additional potential cause for local thrombosis is inherited or acquired susceptibilities to thrombosis. A recent study of 15 patients with retinal artery occlusion showed that increased levels of fibrinogen, lipoprotein (a) and plasma viscosity may play a role in its pathogenesis, perhaps by acting synergistically with hypertension.[3] Hyperhomocysteinaemia has also been described,[4,5] but the small number of patients studied precluded examination of the relation between elevated homocysteine levels, hypertension and carotid atheromas.[5] In other studies of small series of patients and anecdotal case reports, elevated anticardiolipin levels,[6,7] deficiencies of antithrombin, protein C or protein S[8] and presence of factor V Leiden[8] have been observed.

In this study we selected a group of patients with CRAO or BRAO in whom echocardiography and carotid Doppler ultrasound examinations were normal, and examined them for the common vascular risk factors and thrombophilias.

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Patients and methods

The study group consisted of consecutive patients who presented with acute CRAO or BRAO in whom imaging examinations of the carotid arteries and heart excluded an embolic source during the years 1992–1999 at the Sheba and Sapir Medical Centers' Eye Institutes in Israel. The diagnosis of acute CRAO was based on a history of sudden painless visual loss, and findings of afferent pupillary defect, whitening of the retina at the posterior pole with cherry red spot, and delay in arteriovenous transit time observed by fluorescein angiography. The diagnosis of acute BRAO was based on a history of visual disturbance and finding of an area of superficial retinal whitening in the posterior pole along the course of an obstructed artery, and delayed filling of the occluded artery on fluorescein angiography. All patients underwent Doppler ultrasound examination of the carotid arteries, and transthoracic or transoesophageal echocardiography.

Excluded from the study were patients with features consistent with vasculitis, cerebrovascular, hepatic or renal diseases and trauma. Of 25 eligible patients 2 refused to participate in the study, 1 died and 1 was lost to follow-up. After giving their consent, the 21 remaining patients underwent clinical investigation. Particular attention was paid to arterial hypertension (blood pressure more than 160/90 mmHg, diabetes mellitus (patients on glucose-lowering drugs), hypercholesterolaemia (total cholesterol levels 260 mg/dl or above), ischaemic heart disease (patients with documented myocardial infarction or on drugs administered for coronary insufficiency), cardiomyopathy or heart valve dysfunction diagnosed by echocardiography, atrial fibrillation, arterial and venous thromboembolism, and smoking. A thorough neurological examination was carried out.

A control group consisted of 243 healthy subjects; 98 persons belonged to the personnel of the Sheba Medical Center (38 men, 60 women; median age 47 years) and 145 men belonged to the Israel Defense Forces (median age 40 years). The Human Subject Ethics Committee of the Sheba Medical Center approved the study.

Blood was taken from fasting patients in disodium ethylene diamine tetraacetate solution (EDTA) for assessment of homocysteine level in plasma and for DNA extraction that was performed by a standard method.[10] In addition, blood was taken in 129 mM buffered citrate and plasma separated and stored at –35 °C until analysed. Serum separated from clotted whole blood was also used for anticardiolipin titre determination. A complete blood count was performed in each subject. In control subjects DNA was extracted from blood samples sent for routine blood counts. Antithrombin was measured by a chromogenic assay based on inhibition of activated factor X (Chromogenix MöIndal, Sweden). Protein C was determined by a chromogenic assay (Diagnostica Stago Asnieres, France). Free protein S antigen was determined by a commercially available kit (Diagnostica Stago Asnieres, France). Normal ranges (± 2 SD of the mean) observed in healthy subjects for antithrombin, protein C and free protein S were 75–125%, 70–130% and 60–135%, respectively. Lupus anticoagulant was determined using a dilute Russell's Viper venom time (DRVVT)-based assay (Gradipore, Australia). In this assay a ratio is determined between DRVVT of plasma with a phospholipid sensitive to lupus anticoagulant and DRVVT of plasma with a phospholipid insensitive to lupus anticoagulant. Ratios of 1.6 or more and 1.3 or more were considered positive in patients receiving or not receiving warfarin, respectively. Anticardiolipin antibodies (IgG, IgM) were determined by an enzyme-linked immunoabsorbent assay (ELISA) (Selisa, Cambridge Life Sciences, UK).

Homocysteine level was determined by high-pressure liquid chromatography (HPLC) with fluorescence detection, based on a procedure described by Jacobsen *et al*.[11] The normal range of homocysteine levels in healthy subjects was 4–11 μM (± 2 SD). Fibrinogen level was determined according to the Clauss method.[12] The normal range observed in healthy subjects for fibrinogen was 200–400 mg/dl.

Factor V G1691A (Leiden) mutation was detected by polymerase chain reaction (PCR) and MnII digestion as described elsewhere.[13] Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism was detected by PCR and *Hinf*I digestion as described by Frosst *et al*.[14] Factor II G20210A polymorphism was analysed by a slight modification of the originally described method.[15] Subjects were considered 'positive' for factor V G1691A (Leiden), and for the polymorphism of factor II G20210A, if they were heterozygous or homozygous for the respective polymorphisms. Regarding MTHFR C677T, only homozygous subjects were considered positive.

The study and control groups were compared with respect to the potential clinical prothrombotic factors by chi-square tests and odds ratios with 95% confidence intervals (CI).

Results

The patients' characteristics are presented in Table 1. Six women and 5 men had CRAO, and 4 women and 6 men had BRAO. Only one patient presented with bilateral CRAO. The age range of the patients at the time of the event was 18–79 years, with a median of 60 years. Thirteen of the 21 patients (62%) had arterial hypertension, 2 had diabetes mellitus and 2 had hypercholesterolaemia. Twelve patients (57%) had a history of cigarette smoking but only 3 subjects were still smoking at the time of the event. None of the patients had previous episodes of thrombosis or a history of migraine.

Three patients with BRAO had another episode of BRAO in the fellow eye while on aspirin treatment. In 2 of these patients the second event occurred 1 month after the initial event and in the third patient the recurrent event occurred 2 years later. Cerebral artery thrombosis occurred in another patient 2 years after CRAO.

Table 1. *Vascular risk factors and thrombophilia in patients with CRAO and BRAO*

Sex	Age[a] (years)	Initial site of thrombosis	Subsequent site of thrombosis	Vascular risk factors[†]	Thrombophilia[‡]	Homocysteine (μmol/l)	Fibrinogen (mg/dl)
F	64	L-CRAO		Hyp	MTHFR	3.5[b]	476
M	50	R-CRAO		Hyp	FV	9.2	310
M	60	L-CRAO		Hyp	Anticardiolipin	10.2	
F	65	R-BRAO		Hyp	FV, MTHFR	10.9[b]	237
F	60	L-CRAO		Hyp	MTHFR	5.5	310
M	67	L-BRAO	R-BRAO	Hyp, Hych	FV, MTHFR	8.3[b]	335
F	64	L-CRAO		Hyp	MTHFR	4.7	303
M	29	R-CRAO		Hyp	–	4.2	287
F	69	R-CRAO		Hyp	–	7.2	261
M	42	L-BRAO		Hyp	–	7.3	310
M	56	R-CRAO		DM	–	5.2	307
M	77	R-BRAO		Hyp	–	10.4	272
F	48	R-CRAO		Hyp, Hych, DM	–	6.5	
M	79	L- & R-CRAO		Hyp	–	8.4	380
F	68	R-BRAO		–	MTHFR	5.7	307
M	43	R-BRAO			LAC, MTHFR, anticardiolipin	17.6	388
M	33	R-BRAO	L-BRAO	–	–	6.7	272
F	18	R-BRAO	L-BRAO	–	–	4.8	307
M	33	R-BRAO				7.4	320
F	63	R-BRAO		–	–	5.9	350
F	74	L-CRAO			–	6.2	320

CRAO, central retinal artery occlusion; BRAO, branch retinal artery occlusion; L, left; R, right; Hyp, Hypertension; Hych, hypercholesterolaemia; DM, diabetes mellitus; MTHFR, MTHFR 677T homozygosity; FV, FV G1691A (Leiden) heterozygosity; LAC, lupus anticoagulant.
[a]At presentation.
[b]On folate treatment prior to homocysteine measurement.

Of the 21 patients, 9 (43%) had an inherited or an acquired thrombophilia (Table 1). Four patients were homozygotes for MTHFR C677T, 1 was heterozygous for factor V G1691A, 1 had a 27 U/ml titre of an IgM anticardiolipin (normal levels less than 7.6 U/ml), 2 were heterozygous for factor V G1691A and homozygous for MTHFR C677T and 1 had four thrombophilia markers, i.e. lupus anticoagulant, a 31 U/ml titre of IgM anticardiolipin, homozygosity for MTHFR C677T and hyperhomocysteinaemia. Thus, in 3 of the 9 affected patients more than one thrombophilic marker was detected. None of the patients bore factor II G20210A, and none was found to have a deficiency of protein C, protein S or antithrombin. One patient was on warfarin treatment and therefore his protein C and protein S could not be evaluated. Fibrinogen levels were within the normal range in 18 of 19 patients examined, and increased in 1 patient (Table 1).

The prevalence of homozygous MTHFR C677T was significantly higher ($p = 0.015$) in the RAO group than in the control group, yielding an odds ratios of 3.18 (Table 2). The frequency of factor V G1691A was also higher in the RAO group (OR 2.36), but the difference did not reach statistical significance, probably due to the small sample size.

Discussion

In the present study we defined a group of patients with CRAO or BRAO who had no apparent signs of an embolic source and evaluated them for a systemic cause for their local thrombotic event. Sixty-two per cent of the patients had hypertension, 9% had diabetes mellitus and 9% had hypercholesterolaemia. These arteriovascular risk factors observed in 14 of the 21 patients are very common in the population, whereas RAO is rare, which is consistent with the notion that additional risk factors probably play a role in causing RAO. Indeed, 7 of the 14 patients who had a vascular risk factor were found to also have a thrombotic tendency (3 were homozygous for MTHFR C677T, 2 were heterozygous for factor V Leiden, 1 had an increased titre of anticardiolipin and 1 had a double defect consisting of homozygosity for MTHFR C677T and heterozygosity for factor V Leidin: Table 1). Hence, RAO may be precipitated by the joint effect of

Table 2. *Univariate comparison of RAO patients and controls with respect to MTHFR C677T and factor V G1691A*

Prothrombotic marker	RAO ($n = 21$) Affected	RAO ($n = 21$) % affected	Controls ($n = 243$) Affected	Controls ($n = 243$) % affected	p	Odds ratio	95% CI
MTHFR C677T[a]	7	33.3	33	13.6	0.015	3.18	1.20–8.47
Factor V G1691A[b]	3	14.3	16	6.6	0.190	2.36	0.63–8.88

RAO, retinal artery occlusion.
[a]Homozygotes.
[b]Heterozygotes.

vasculopathic and thrombogenic factors. Still, for the remaining 7 patients with vascular risk factors no additional risk factors were identified, which may suggest that the vasculopathic effects of hypertension, diabetes and hypercholesterolaemia are sufficiently powerful under certain circumstances, or that there are as yet unidentified thrombotic or other risk factors. Also of interest in this regard are 2 patients in whom only thrombogenic factors were detectable, i.e. 1 with MTHFR C677T homozygosity, and 1 with lupus anticoagulant, an elevated titre of anticardiolipin and MTHFR C677T homozygosity, and 3 patients who had neither vasculopathic nor thrombogenic risk factors albeit recurrent BRAO was observed in 2 of them (Table 1).

In view of the small study group, analysis of the prevalence of the various thrombophilic markers has its limitations. Nevertheless, MTHFR C677T homozygosity was significantly more frequent in patients (33%) than in the controls (14.3%), yielding an odds ratio of 3.18 (Table 2). This genotype has been shown to be associated with a 50% reduction of the specific activity of MTHFR and with elevated levels of serum homocysteine,[14] and hyperhomocysteinaemia was reported in associated with RAO.[5,16] In our series, however, we observed only one homozygotye for MTHFR C677T in whom the level of homocysteine was increased. Since folic acid intake strongly affects homocysteine levels,[17] it is possible that at the time of RAO hyperhomocysteinaemia was present due to nutritional deficiency of folic acid, whereas at the time of testing the level was normal due to appropriate folic acid intake. Three of the patients were taking 5 mg folic acid per day when tested.

Factor V Leiden was also more frequent in the study group but the difference did not reach statistical significance, albeit yielding an odds ratio of 2.36. Anecdotal cases have described an association between RAO and the presence of factor V Leiden,[9,18,19] but a series of 29 cases failed to show an increased prevalence of factor V Leiden.[20]

Increased levels of fibrinogen and lipoprotein (a) were recently found in patients with retinal artery occlusion.[3] We detected an increased fibrinogen level only in 1 of 19 patients examined and the mean level of the whole group (313 mg/dl) did not differ significantly from the mean value of healthy subjects. Clearly, more patients need to be studied in order to clarify the discrepant findings.

In conclusion, three relatively frequent types of thrombophilia were found in 43% of patients with retinal artery occlusion in whom no embolic source was demonstrated by imaging examinations. A search for thrombophilia in such patients is advisable even when vascular risk factors are identified, as there appears to be an interaction between vasculopathic and thrombogenic risk factors. At this stage, no therapeutic recommendations can be made except for giving folic acid to patients with homozygosity for MTHFR C677T and/or hyperhomocysteinaemia.

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