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Atherosclerotic and thrombophilic risk factors in patients with recurrent central retinal vein occlusion

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PURPOSE. *Atherosclerotic and thrombophilic risk factors may be causes of central retinal vein occlusion (CRVO). The aim of this study was to evaluate the prevalence of the aforesaid risk factors in patients with recurrent CRVOs and patients with a single episode of CRVO.*
METHODS. *Seventeen patients with recurrent CRVO and 30 with a single episode of CRVO were enrolled. The atherosclerotic risk factors investigated were hypertension, diabetes, smoking, and dyslipidemia. Specific laboratory tests for the following thrombophilic markers were performed: homocystinemia (Hcy), lipoprotein (a), factor VIII, factor II G20210A and factor V G1691A polymorphisms, lupus anticoagulant, anticardiolipin antibodies, plasminogen activator inhibitor-1, and deficit of vitamins B6, B12, and folic acid. A multivariate analysis, adjusted for age, gender, traditional and thrombophilic risk factors, was performed. Statistical significance was set at $p \leq 0.05$.*
RESULTS. *Hypercholesterolemia, hypertriglyceridemia, fasting, and postmethionine hyperhomocysteinemia (HHcy) were more prevalent in recurrent CRVO patients ($p<0.001$, $p<0.001$, $p=0.006$, and $p=0.005$, respectively). At multivariate analysis, hypercholesterolemia (OR: 5.04, 95% CI 1.39-18.17; $p=0.025$), hypertriglyceridemia (OR: 5.60, 95% CI 1.52-20.61; $p=0.017$), fasting HHcy (OR: 5.77, 95% CI 1.39-23.89; $p=0.028$), and postmethionine HHcy (OR: 10.88, 95% CI 2.50-47.42; $p=0.002$) were found to be significantly associated with recurrent CRVO.*
CONCLUSIONS. *Dyslipidemia and hyperhomocysteinemia are independent risk factors for the occurrence of recurrent CRVO. A complete assessment of atherosclerotic and thrombophilic risk factors is recommended in CRVO patients. In addition, the need for a specific treatment is suggested. (Eur J Ophthalmol 2008; 18: 233-8)*

KEY WORDS. *Central retinal vein occlusion, Dyslipidemia, Homocysteine, Thrombophilia*

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INTRODUCTION

Central retinal vein occlusion (CRVO) is a common vascular disease, especially among the elderly (1, 2). The risk factors implied in the occurrence of this disease are not completely known. Although CRVO has a number of risk factors in common with atherosclerosis, traditional atherosclerotic risk factors are not sufficient to explain the pathogenesis of the disease in all affected patients (3-6). In recent years, a growing body of evidence supported an association between thrombophilia and hypofibrinolysis and the occurrence of CRVO, especially in younger individuals (7-16). Conversely, other studies did not show a significant correlation between thrombophilic risk factors and CRVO (17-19). Nevertheless, there are meta-analysis in which hyperhomocysteinemia is identified as a marker of increased risk of developing CRVO (20, 21).
Recurrent CRVOs, both unilateral and bilateral, have been found to be associated with various systemic diseases (22-27). Bilateral CRVOs are relatively frequent and often

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concomitant, in cases of malignancies or other diseases leading to a hyperviscosity syndrome, but can occur also in subjects free from this pathologic condition (23, 25-27). In fact, in the literature there are case reports and small series of patients usually revealing the presence of separate thrombophilic markers associated or not with cardiovascular risk factors in this particular clinical pattern of CRVO (28-33).

The aim of the present study was to compare the prevalence of the traditional cardiovascular and thrombophilic risk factors in a group of patients with recurrent CRVO and a group of patients with a single episode of CRVO.

MATERIALS AND METHODS

Study population

The study population comprised 47 consecutive patients with a clinical diagnosis of nonischemic CRVO who had been referred to the Eye Clinic of the University of Florence, Italy. The patients were divided in two groups: 17 with recurrent CRVO in the same or in the contralateral eye within 6 months from the first episode and 30 with a single episode of CRVO in a 5-year follow-up period. Of the 17 patients with recurrent CRVO, 10 (58.82%) presented with bilateral CRVO and 7 (41.18%) with recurrent CRVO in the same eye. The study complied with the tenets of the Declaration of Helsinki and of the local ethics committee. All the participants gave signed informed consent before the enrollment.

All the patients underwent a complete physical and ophthalmic examination. A detailed interview addressed to personal history of cardiovascular diseases and risk factors was performed. Patients with systemic conditions known as causes of hyperviscosity syndrome were excluded from the study, as well as patients with ocular pathologies other than CRVO and having had previous ocular surgery. Echocardiogram, electrocardiogram, and color Doppler imaging of the epiaortic vessels were performed in all patients and excluded heart diseases and hemodynamically significant carotid artery stenosis in the whole study population. All the subjects underwent orbital echography and color Doppler imaging of the retrobulbar vessels to exclude the presence of local factors potentially leading to recurrent CRVO.

The subjects were classified as having arterial hypertension according to the guidelines of the European Society of Hypertension/European Society of Cardiology (34) or if they reported taking antihypertensive medications, as verified by the interviewer. Diabetic subjects were defined in line with the American Diabetes Association (35) or on the basis of self-report data (if confirmed by medication or chart review). Dyslipidemia was defined following the criteria of the ATP III Expert Panel of the US National Cholesterol Education Program (36). Current smoking status was determined at the time of physical examination.

Laboratory measurements

Venous blood was collected from the basilic vein after an overnight fasting between 08:00 AM and 08:30 PM. Cholesterol and triglyceride values were obtained by means of enzymatic and colorimetric methods, respectively. For determining postmethionine homocystinemia, 100 mg/Kg of body weight of L-methionine was administered in approximately 200 mL of fruit juice after the fasting blood sampling and a second blood sample was obtained after 4 hours. Whole venous blood was collected in tubes containing ethylenediaminetetraacetate (EDTA) 0.17 mol/L, immediately put in ice, and centrifuged within 30 minutes at 4 °C (1500 *g* × 15 min). Plasma samples were stored at –20 °C until assay. Homocysteine (Hcy) levels were determined by a Fluorimetric Polarized Immuno Assay (FPIA method, IMX Abbott Laboratories, Oslo, Norway). High levels of fasting and postmethionine Hcy were diagnosed when values exceeded the 95th percentile of distribution in controls (fasting: men 19 mmol/L, women 13 mmol/L; postmethionine: men 38 mmol/L, women 35 mmol/L). To determine plasminogen activator inhibitor-1 (PAI-1), factor VIII (FVIII), and lupus anticoagulant (LA), blood was drawn directly into evacuated tubes containing sodium citrate 0.129 M, preserved, centrifuged, frozen, and stored at –80 °C. PAI-1 antigen levels were determined by an ELISA (Asserachrom, Stago, France). FVIII levels were determined by a clotting method (Dade Behring, Germany). PAI-1 and FVIII levels above the 95th percentile of distribution in controls were considered over the normal range (40 ng/mL and 150%, respectively). Platelet poor plasma (PPP) for LA test was obtained by centrifuging blood samples and was stored at –80 °C until used. The tests chosen to detect the presence of LA were 1) diluted (1:50) aPTT (Pathromptin, Dade Behring); 2) Kaolin Clotting Time (KCT, Stago); 3) Tissue thromboplastin inhibition test (TITT, Dade Behring, using 1:1000 dilution); 4) diluted Russell's Viper Venom Time (dRVVT; IL test LAC screen-

ing, Instrumentation Laboratory, Milan, Italy). Mixing studies with normal plasma (pooled PPP from 20 normal subjects) were employed to exclude clotting factor deficiencies or the presence of antibodies against specific coagulation proteins. Samples found to be abnormal were also assayed according to the platelet neutralization procedure (PNP, Stago) as the confirmation test. We considered LA positive those patients with tests confirmed by PNP. Sera for testing anticardiolipin antibodies (aCL) and lipoprotein (a) [Lp(a)] were obtained by centrifuging blood collected in evacuated tubes without anticoagulant and stored at –20 °C. The aCL assay was performed by an ELISA (First Cardiolipin IgM and IgG, Eurospital, Trieste, Italy) and aCL levels were reported in MPL units (for IgM) and GPL units (for IgG). On the basis of analysis of several hundred normal serum specimens performed in our laboratory in the past, and according to the literature, values >40 U for both IgM and/or IgG were considered abnormal. Lp(a) levels were determined by an ELISA assay (Apo[a] ELISA, Mercodia). Lp(a) levels >300 mg/L were considered over the normal range. For the detection of the G1691A polymorphism of the factor V (FV) gene and the G20210A polymorphism of the factor II gene, genomic DNA was extracted from peripheral blood-cell lymphocytes, amplified by polymerase chain reaction, and then digested wit *Mn*lI and *Hind*III restriction enzymes, respectively. To determine circulating vitamins (B6, folic acid, and B12) venous blood was put in tubes without anticoagulant and centrifuged at room temperature (2000 *g* × 15 min). Sera samples were stored at –20 °C until assay. Sera levels of vitamin B6 were determined by using a commercial HPLC assay with fluorescence detection (Immundiagnostik AG, Bensheim, Germany) whereas sera levels of folic acid and vitamin B12 were measured by radioimmunoassay (ICN Pharmaceuticals, Orangeburg). Normal range for vitamin B6, folic acid, and vitamin B12 blood values were 3–18 ng/mL, 3–17 ng/mL, and 180–970 pg/mL, respectively.

Statistics

Results are shown as mean ± standard deviation (SD). Statistical comparisons between the two groups of patients were carried out by means of Student *t*-test for unpaired data and chi-square test. In order to investigate the possible association between atherosclerotic and thrombophilic risk factors and recurrent CRVO, a logistic regression analysis was performed after adjustment for age, gender, smoking, dyslipidemia, hypertension, diabetes, and thrombophilic markers. All odds ratios (ORs) are given with their 95% confidence intervals (CIs). Statistical significance was set at $p<0.05$.

RESULTS

Demographic characteristics of the study population and prevalence of traditional atherosclerotic risk factors in the two groups of patients are outlined in Table I.

Cholesterol and triglyceride blood values were found to be significantly higher in patients with recurrent CRVO than in those with a single episode of CRVO (210.10±36.77 mg/dL vs 172.70±33.56 mg/dL, $p<0.001$, and 173.40±41.48 mg/dL vs 135.50±22.60 mg/dL, $p<0.001$, respectively). None of the participants had diabetes.

Among the thrombophilic risk factors, both fasting and postmethionine hyperhomocysteinemia (HHcy) were sig-

TABLE I - CHARACTERISTICS OF THE PATIENTS ENROLLED AND PREVALENCE OF CARDIOVASCULAR RISK FACTORS

	Recurrent CRVO	Single episode CRVO	p value
Number	17	30	
Age, yr	69.94±10.05	67.43±7.95	0.351
Gender	8 men/9 women	17 men/13 women	0.741
Hypertension, n (%)	11 (64.70)	14 (46.66)	0.375
Smoking habit, n (%)	3 (17.64)	4 (13.33)	0.978
Hypercholesterolemia, n (%)	11 (64.70)	8 (26.66)	0.025
Hypertriglyceridemia, n (%)	12 (70.58)	9 (30.00)	0.017

CRVO = Central retinal vein occlusion

TABLE II - PREVALENCE OF THROMBOPHILIC RISK FACTORS

	Recurrent CRVO	Single episode CRVO	p value
Fasting HHcy	8 (47.05)	4 (13.33)	0.028
Postmethionine HHcy	14 (82.35)	9 (30.00)	0.002
Elevated PAI-1 levels	0 (0)	4 (13.33)	0.303
Elevated FVIII levels	2 (11.76)	5 (16.66)	0.978
LA positive	4 (23.52)	6 (20.00)	0.931
Elevated aCL levels	6 (35.29)	7 (23.33)	0.588
Elevated Lp(a)	5 (29.41)	11 (36.66)	0.854
G1691A polymorphism	0 (0)	2 (6.66)	0.737
G20210A polymorphism	0 (0)	3 (10.00)	0.467
Reduced vitamin B6 levels	1 (5.88)	2 (6.66)	0.606
Reduced folic acid levels	2 (11.76)	4 (13.33)	0.764
Reduced vitamin B12 levels	0 (0)	1 (3.33)	0.771

Values are n (%).
CRVO = Central retinal vein occlusion; HHcy = Hyperhomocysteinemia; PAI-1 = Plasminogen activator inhibitor-1; FVIII = Factor VIII; LA = Lupus anticoagulant; aCL = Anticardiolipin antibodies; Lp(a) = Lipoprotein (a)

TABLE III - INDEPENDENT RISK FACTORS FOR RECURRENT CENTRAL RETINAL VEIN OCCLUSION AT MULTIVARIATE ANALYSIS (adjusted for age, sex, and cardiovascular and thrombophilic risk factors)

	Odds ratio (95% CI)	p value
Hypercholesterolemia	5.04 (1.39–18.17)	0.025
Hypertriglyceridemia	5.60 (1.52–20.61)	0.017
Fasting HHcy	5.77 (1.39–23.89)	0.028
Post-methionine HHcy	10.88 (2.50–47.42)	0.002

HHcy = Hyperhomocysteinemia

nificantly more frequent in patients with recurrent CRVO (16.40±6.30 vs 12.46±3.17 µmol/L, p=0.006, and 38.60±14.29 vs 28.76±8.54 µmol/L, p=0.005, respectively). The prevalence of the thrombophilic and hypofibrinolytic markers in the two study groups is summarized in Table II.

On multivariate analysis, adjusted for age, gender, and traditional cardiovascular and thrombophilic alterations, independent risk factors associated with the occurrence of recurrent CRVO were hypercholesterolemia, hypertriglyceridemia, and hyperhomocysteinemia, both fasting and/or postmethionine, which determined a fivefold to tenfold increase in the odds of having recurrent CRVO (Tab. III).

DISCUSSION

The present study compares a group of patients with recurrent CRVO and a group with a single episode of CRVO. Our results suggest that dyslipidemia and hyperhomocysteinemia are associated with an increased risk of having recurrent CRVO.

Many studies have demonstrated that patients with CRVO are more likely to have atherosclerotic or thrombophilic alterations than healthy controls (3-15). In particular, HHcy has been consistently demonstrated as a factor strongly associated with an increased risk of developing CRVO (7, 8, 10-13, 16).

Hypertension, hyperhomocysteinemia, and some other thrombophilic alterations have been shown to be present in case reports and small case series of patients with recurrent CRVO (22, 28-33).

To our knowledge, the present study is the first one evaluating the prevalence of the major well-known risk factors at the same time in subjects with recurrent CRVOs and subjects with a single episode of CRVO. Our findings are in agreement with those reported by Blondel et al, who observed a higher prevalence of HHcy in bilateral CRVOs (11). As for other risk factors mentioned in some case reports, our study does not confirm that antiphospholipid antibody syndrome or factor V Leiden mutation may be causes of recurrent CRVO (29, 31, 32). Our results are in line with those by authors who reported only hyperhomocysteinemia, but not other thrombophilic risk factors, as

marker of augmented risk of CRVO occurrence (20, 21). Conversely, the present findings are at variance with those by authors who denied a role even for hyperhomocysteinemia in the pathogenesis of CRVO (17-19).

In our study, dyslipidemia and HHcy were significantly associated with the recurrence of CRVO. Therefore, a complete assessment of these risk factors is recommended in all the individuals presenting with a first episode of CRVO. Our findings may have relevant therapeutic implications. In fact, the correction of dyslipidemia may be obtained by environmental and/or pharmaceutical interventions, while homocysteine levels may be successfully reduced by vitamin supplementation. Furthermore, a long-lasting antithrombotic treatment should be initiated. All these therapeutic measures seem to be reasonable in patients presenting with CRVO, in order to reduce the risk of recurrent retinal vein occlusions. The small sample size is a clear limitation of the present study. In addition, the presence of several clinical variables could be confounding in the interpretation of the results. Therefore, further investigations, conducted on larger and possibly more homogeneous populations, are needed to confirm our preliminary data. Moreover, prospective studies would be suitable to evaluate the efficacy of the secondary prevention on avoiding the recurrence of central retinal vein occlusion.

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