Plasma Homocysteine Concentrations in Acute and Convalescent Changes of Central Retinal Vein Occlusion in a Chinese Population

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Submitted: February 23, 2014
Accepted: May 19, 2014

Citation: Dong N, Xu B, Tang X. Plasma homocysteine concentrations in acute and convalescent changes of central retinal vein occlusion in a Chinese population. *Invest Ophthalmol Vis Sci*. 2014;55:4057–4062. DOI:10.1167/iovs.14-14226

Purpose. Homocysteine is a potential risk factor for central retinal vein occlusion (CRVO), but this remains controversial. We measured fasting total plasma homocysteine (tHcy) concentrations immediately after CRVO and in the convalescent period to investigate this controversy.

Methods. We measured fasting tHcy concentrations in 36 consecutive patients with CRVO within three days; and at 1, 3, and 6 months after CRVO; and once in 36 control subjects. The vitamin B_{12} and folate levels, and the presence of C677T *MTHFR* polymorphisms, were analyzed in all patients and controls.

Results. Median tHcy concentrations were not significantly higher than in matched control subjects in the acute phase of CRVO (9.66 [10.75 ± 4.09] vs. 9.25 [9.96 ± 4.02] μmol/L, $P = 0.371$) and 1 month after CRVO ($P = 0.119$). However, tHcy levels increased significantly in the convalescent period and were significantly higher than in control subjects at 3 CRVO ($P = 0.010$) and 6 ($P < 0.001$) months after CRVO. Furthermore, tHcy levels of the ischemic CRVO patients at 6 months after CRVO were significantly higher than in nonischemic CRVO patients ($P = 0.028$). However, these observations did not appear to be explained by alteration in serum folate, vitamin B_{12} concentrations, and the *MTHFR* C677T genotype.

Conclusions. The tHcy levels are not immediately elevated after CRVO, but increase in the convalescent period. These data do not support the hypothesis that raised tHcy concentrations are independent risk factor for CRVO. Instead, it is possible that elevated tHcy levels may be caused by the disease process itself.

Keywords: acute phase, convalescent period, central retinal vein occlusion, *MTHFR* C677T, total plasma homocysteine

Central retinal vein occlusion (CRVO) is one of the most common vision-threatening retinal vascular diseases; it affects primarily elderly patients, resulting in macular edema, vitreous hemorrhage, and neovascular glaucoma.[1] Elevated total plasma homocysteine (tHcy), which is associated with venous thrombosis and cardiovascular disease, has been suggested as an important potentially modifiable risk factor for atherosclerosis.[2–5] Similarly, several studies have demonstrated elevated tHcy levels to be a potential risk factor in CRVO.[6–13] However, not all studies demonstrate a clear relationship between elevated tHcy levels and CRVO.[14–20] Thus, the role of tHcy still is being debated.

One potential limitation of the previous studies is that CRVO patients were recruited at variable intervals after the disease onset. Keeping in mind that tHcy concentrations may change over time, we explored the relationship between plasma homocysteine concentrations and CRVO in the acute phase to control the confounding of which venous blood samples were collected at different time points after CRVO.[20] Our previous studies showed no association between tHcy in the acute phase after CRVO and CRVO occurrence in a Chinese population.[20] However, it now is widely accepted in clinical practice that plasma tHcy levels are notoriously labile. The recorded plasma tHcy levels in acute and convalescent changes of CRVO have not been elucidated, to the best of our knowledge.

Homocysteine, a potentially cytotoxic sulfur-containing amino acid, is an intermediate product of methionine metabolism.[13] After its formation, homocysteine may be remethylated to methionine by the enzyme, 5,10 methyltetrahydrofolate reductase (MTHFR), which requires folate and vitamin B_{12} as cofactors.[13] Homozygosity for the C677T mutation in the MTHFR enzyme causes a 50% decrease in enzyme activity, leading to mild hyperhomocysteinemia.[21,22] Thus, a mild increase in tHcy can be caused by various factors, such as low folate intake, inadequate plasma concentrations of B vitamins, and genetic factors.

Therefore, we explored plasma homocysteine concentrations, vitamin B_{12}, and folate in acute and convalescent changes CRVO in a Chinese population. In addition, in light of the overwhelming evidence that elevated tHcy is related to homozygosity for the C677T mutation in the MTHFR enzyme, we explored the *MTHFR* C677T genotype in the Chinese population.

Materials and Methods

Study Design and Procedure

The present study used a matched case–control design, recruiting participants between April 2011 and January 2013. The study was approved by the Ethics Committee of Beijing Shijitan Hospital, Capital Medical University, Beijing, People's Republic of China, and was performed in accordance with the Declaration of Helsinki. Each subject received a detailed information leaflet and provided informed written consent before participation.

Consecutive patients with CRVO were included only if they could describe accurately the occurrence time of the decreased visual acuity and/or visual field defect, to ensure that all patients were tested within three days after CRVO. The age- and sex-matched controls were recruited from our department and were free of retinal disease, such as retinal vascular occlusion, anterior ischemic optic neuropathy, and vasculitis. Each patient was matched with one control subject. All patients and controls were recruited from the same narrow geographic area (Beijing and surroundings) to control for dietary habits. Patients with branch or hemispheric retinal vein occlusion, or coexistent central or branch retinal artery occlusion were excluded from the case group. Additional exclusion criteria for cases and controls included major systemic illness, vasculitis, renal, hepatic, thyroid, or cardiovascular diseases, and current medication with vitamin B_{12}, B6, or folic acid. All patients and controls with malignant hematologic diseases (chronic leukemias, polycythemia, Waldenström's macroglobulinemia, multiple myeloma) or other tumors also were excluded.

Patients were subjected to a complete ophthalmologic examination and general physical examination, including best-corrected visual acuity (BCVA), relative afferent pupillary defect (RAPD), electroretinogram (ERG), slit-lamp–assisted biomicroscopy of the anterior segment, optical coherence tomography (OCT), and fundus examination, which was used for the clinical diagnosis of CRVO. The diagnosis of CRVO was made when ophthalmoscopic fundus examination revealed disc swelling, venous dilation or tortuosity, and retinal hemorrhages in all quadrants of the retina. The CRVO patients were divided into a nonischemic CRVO group and an ischemic CRVO group based on fluorescence fundus angiography (FFA). All patients underwent the above examination at entry (within three days after CRVO), and at 1 (30 ± 3 days), 3 (90 ± 7 days), and 6 (180 ± 7 days) months after CRVO.

Subjects with blood pressure above 140/90 mm Hg or who were receiving any antihypertensive medication were considered hypertensive. Hypercholesterolemia was defined as fasting total plasma cholesterol levels above 200 mg/dl. Hypertriglyceridemia was classically defined as fasting plasma triacylglycerols (triglycerides [TG]) above 200 mg/dl. Subjects were defined as current smokers or nonsmokers (had not smoked within the last 1 year).

The total plasma homocysteine, vitamin B_{12}, and folate levels, and the presence of *MTHFR* C677T polymorphisms were analyzed in blood samples that were collected from patients and controls in the morning after an overnight fast of at least 8 hours. Fasting venous blood samples were collected from patients at entry (within three days after CRVO), and 1 (30 ± 3 days), 3 (90 ± 7 days), and 6 (180 ± 7 days) months after CRVO.

Measurement of Plasma tHcys

The method of measurement of plasma tHcys has been described in detail previously.[20] In brief, venous blood samples for homocysteine measurement were drawn into EDTA-containing tubes, transported on ice, and immediately centrifuged at 3000*g* for 10 minutes at 4°C, and stored at −70°C until analysis. Measurements of plasma homocysteine were performed using HPLC as described by Ubbink et al.[23] Hyperhomocysteinemia was defined as a total plasma homocysteine level exceeding the 95th percentile of controls.[10,24]

Measurement of Vitamin B_{12} and Folate

The method of measurement of vitamin B_{12} and folate has been described in detail previously.[20] In brief, serum vitamin B_{12} levels were quantified with an Abbott AxSYM analyzer using a Microparticle Enzyme Immunoassay (Abbott Laboratories, Abbott Park, IL, USA). These data are reported as pg/mL. The levels of serum folate were quantified using an automated chemiluminescence system (Bayer Health Care, Leverkusen, Germany). The levels of folate are reported as ng/mL. Normal values for plasma vitamin B_{12} and folate are 179 to 1096 pg/mL and 2.6 to 32 ng/mL, respectively.

MTHFR C677T Polymorphism

The method of *MTHFR* C677T polymorphism has been described in detail previously.[20] In brief, molecular genetic analysis of the 677C-T mutation in the *MTHFR* gene was performed in patients and controls. A fragment of the *MTFHR* gene was amplified by PCR (sense 5′-TGAAGGA GAAGGTGTCTGCGGGA-3′ and antisense 5′-AG GACGGTGCGGTGAGAGTG-3′). The reaction conditions were as follows: initial denaturation for 5 minutes at 96°C; 45 cycles of denaturation for 30 seconds at 93°C, annealing for 30 seconds at 55°C, and extension for 30 seconds at 72°C; and final extension for 7 minutes at 72°C. The amplified DNA was digested with the restriction enzyme Hinf I at 37°C for 3 hours and then run on polyacrylamide gels to determine genotype, as described by Frosst et al.[22]

Statistical Analysis

Data were recorded as the means ± SD or the median and range. The statistical analyses were performed using the program SPSS for Windows Version 17.0 (SPSS, Inc., Chicago, IL, USA). The Pearson χ^2 test was used to compare the proportions of qualitative variables. Student's *t*-test and the Mann-Whitney *U* test were used to compare the means of the quantitative variables between two independent groups. The Kruskal-Wallis test was used to compare multiple groups. A *P* value less than 0.05 was accepted as statistically significant.

Results

Table 1 shows the demographic and clinical characteristics of patients included in the CRVO and control groups. At the final follow-up examination, 36 patients with CRVO (17 males and 19 females) and 36 control subjects (17 males and 19 females) completed 6 months of follow-up. The mean age was 60.6 ± 6.3 years (range, 54–72 years) in patients with CRVO and 60.6 ± 6.3 years (range, 54–72 years) in controls, respectively. Subjects and controls were well matched for age and sex. A history of hypertension, diabetes mellitus, and smoking was more frequent in patients than in controls, but these differences did not reach statistical significance. There were no significant differences in cholesterol or triglyceride levels between subjects and controls.

The tHcy levels of the CRVO patients at four different time points and of controls are given in Table 2. There were no

Table 1. Baseline Characteristics of Patients With CRVO and Controls [*n* (%)]

Characteristics	CRVO	Controls	*P*
N	36	36	1.000*
Sex			
Male	17 (47.2)	17 (47.2)	1.000*
Female	19 (52.8)	19 (52.8)	
Age	60.6 ± 6.3	60.6 ± 6.3	1.000†
Hypertension	20 (55.5)	13 (36.1)	0.098*
Diabetes mellitus	5 (13.9)	4 (11.1)	0.722*
Hypercholesterolemia	9 (25.0)	7 (19.4)	0.571*
Hypertriglyceridemia	8 (22.2)	5 (13.9)	0.358*
Stroke	0 (0.0)	0 (0.0)	1.000*
Myocardial infarction	0 (0.0)	0 (0.0)	1.000*
Smoking	19 (52.8)	12 (33.3)	0.096*

* Pearson χ^2 test.
† Student's *t*-test.

significant differences in mean plasma tHcy between controls and CRVO patients at entry ($P = 0.371$) and 1 month after CRVO ($P = 0.119$). As shown in Figure 1, the tHcy levels gradually increased from acute to convalescent phases of CRVO in CRVO patients. The tHcy levels of the CRVO patients at 3 ($P = 0.010$) and 6 ($P < 0.001$) months after CRVO were significantly higher than in controls.

Sex and age showed a positive correlation with tHcy at all time points ($P < 0.05$) in CRVO patients. The tHcy levels of the female CRVO patients were significantly lower than those of male CRVO patients at all time points. In addition, the tHcy levels of the CRVO patients over 60 years old were significantly higher than those of patients less than 60 years old at all time points. The tHcy levels of the CRVO patients with hypertension and diabetes mellitus were increased than those of CRVO patients without hypertension and diabetes mellitus at all time points; however, there were no significant difference. Presence or absence of hypercholesterolemia, hypertriglyceridemia, and smoking did not affect tHcy levels.

Of the 36 patients with CRVO, there were 17 with ischemic CRVO and 19 with nonischemic CRVO at entry (Table 2). In patients with nonischemic CRVO and good VA (better than 20/40), no immediate therapy had to be advised, but is of importance to prevent complications by controlling vascular risk factors, such as hypertension, diabetes, hypercholesterolemia, and hypertriglyceridemia. Two nonischemic CRVO patients with macular edema were managed by intravitreal triamcinolone acetonide (TA), 17 patients with ischemic CRVO were managed by panretinal photocoagulation (PRP) in an attempt to block the development of ocular neovascularization, and three of 17 patients with ischemic CRVO were administered an intravitreal anti-VEGF agent (Lucentis; Novartis Pharmaceuticals, Landisville, PA, USA) in association with PRP. None of the CRVO patients was administered any drugs affecting the measurement of tHCY, vitamin B_{12}, and folate during the entire follow-up.

The tHcy levels of the ischemic CRVO patients and nonischemic CRVO patients were not statistically significantly different at entry ($P = 0.211$), and 1 ($P = 0.066$) and 3 ($P =$

Table 2. Plasma Homocysteine Levels (μmol/L) in CRVO Patient Groups Characterized by Different Factors

Characteristics	No	At Entry	1 Mo	3 Mo	6 Mo
Sex					
Male	17	12.40 (12.61 ± 4.85)	12.31 (12.57 ± 3.65)	12.68 (12.59 ± 4.68)	12.79 (12.68 ± 4.96)
Female	19	9.11 (9.08 ± 2.34)*	9.08 (9.05 ± 3.04)*	9.13 (9.62 ± 2.57)*	9.25 (9.58 ± 2.66)*
Age, y					
≤60	12	8.78 (8.89 ± 4.05)	9.06 (9.15 ± 3.14)	9.11 (9.52 ± 3.62)	9.12 (9.68 ± 3.69)
>60	24	12.45 (12.88 ± 4.63)*	12.47 (12.42 ± 3.33)*	12.78 (12.75 ± 4.98)*	12.87 (12.86 ± 4.95)*
Hypertension					
Yes	20	10.93 (11.12 ± 3.65)	11.20 (10.89 ± 4.25)	11.63 (11.36 ± 5.12)	11.38 (11.21 ± 5.21)
No	16	10.86 (11.36 ± 4.26)	10.88 (11.09 ± 3.87)	11.01 (11.26 ± 3.12)	10.64 (11.11 ± 7.46)
Diabetes mellitus					
Yes	5	11.06 (11.33 ± 3.16)	11.23 (11.35 ± 4.56)	11.11 (11.27 ± 3.25)	12.13 (11.13 ± 5.23)
No	31	10.89 (11.10 ± 3.53)	10.98 (11.22 ± 3.78)	11.02 (11.05 ± 5.78)	11.09 (11.31 ± 3.46)
Hypercholesterolemia					
Yes	9	11.21 (11.18 ± 3.46)	10.79 (11.03 ± 4.68)	12.01 (11.86 ± 5.26)	11.88 (11.13 ± 6.14)
No	27	11.16 (10.67 ± 6.76)	11.03 (11.23 ± 3.65)	11.15 (11.36 ± 2.64)	10.86 (11.15 ± 4.19)
Hypertriglyceridemia					
Yes	8	11.55 (10.89 ± 3.16)	11.24 (11.56 ± 3.09)	11.03 (11.46 ± 5.12)	11.14 (11.36 ± 3.76)
No	28	10.87 (11.03 ± 4.32)	10.68 (11.10 ± 2.78)	11.36 (11.23 ± 4.26)	10.81 (10.98 ± 5.69)
Smoking					
Yes	19	11.12 (11.23 ± 3.26)	11.09 (11.25 ± 4.22)	12.10 (11.06 ± 5.12)	11.13 (11.42 ± 2.76)
No	17	10.85 (11.03 ± 5.26)	11.12 (11.05 ± 6.16)	10.91 (11.15 ± 3.13)	11.32 (10.98 ± 6.88)
Clinical subgroup					
Ischemic		11.24 (11.25 ± 3.58)	12.40 (12.73 ± 2.89)	13.16 (14.44 ± 4.32)	14.36 (14.57 ± 2.92)*
Nonischemic		9.11 (10.29 ± 4.56)	10.66 (10.52 ± 4.47)	10.01 (11.64 ± 5.22)	12.09 (12.01 ± 4.28)
All patients	36	9.66 (10.75 ± 4.09)	11.32 (11.29 ± 3.99)	12.45 (13.04 ± 4.93)*	13.23 (13.44 ± 3.76)*
Control	36	9.25 (9.96 ± 4.02)			

Values are medians (mean ± SD).
* $P < 0.05$ (Mann-Whitney *U* test).

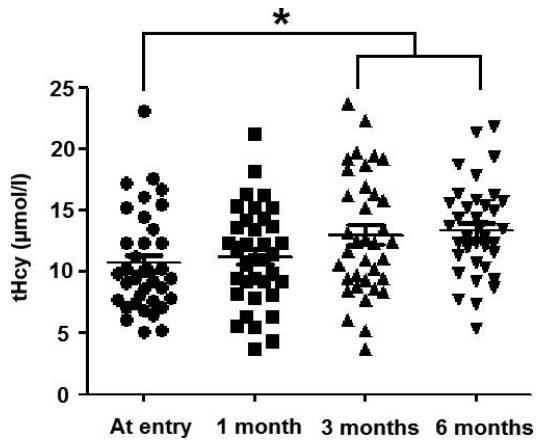

FIGURE 1. Distributions of total plasma homocysteine concentrations in the acute and convalescent phases of central retinal vein occlusion. The results show that the tHcy levels gradually increased from acute phases to convalescent phases of CRVO (*$P < 0.05$).

0.071) months after CRVO. However, the tHcy levels of the ischemic CRVO patients at 6 months ($P = 0.028$) after CRVO were significantly higher than those in nonischemic CRVO patients (Table 1). As shown in Figure 2, the tHcy levels gradually increased from acute to convalescent phases in ischemic CRVO patients ($P < 0.05$). Furthermore, the tHcy levels gradually increased from acute to convalescent phases in nonischemic CRVO patients, but there were no significant differences ($P > 0.05$, Fig. 3). Hyperhomocysteinemia was defined as a total plasma homocysteine level exceeding the 95th percentile of controls (male = 15.5 μmol/L, female = 13.5 μmol/L). Accordingly, eight patients (22.2%) with CRVO and three controls (8.3%) had hyperhomocysteinemia ($P = 0.101$) at entry. Overall, five patients (29.4%) in the ischemic group and three (15.8%) in the nonischemic group had hyperhomocysteinemia ($P = 0.326$) at entry. At the 3-month follow-up examination, one patient with nonischemic CRVO (5.3%) converted to ischemic CRVO. At the final follow-up examination, three patients with nonischemic CRVO (15.8%) converted to ischemic CRVO. Interestingly, all of these three patients were male patients and tHcy levels were higher than 15.5 μmol/L, which is defined as hyperhomocysteinemia.

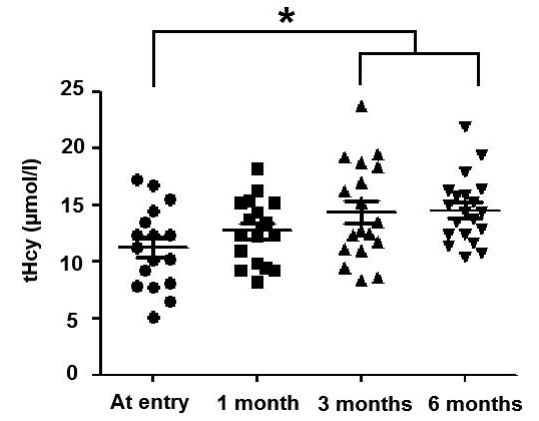

FIGURE 2. Distributions of total plasma homocysteine concentrations in the acute and convalescent phases of ischemic central retinal vein occlusion. The results show that the tHcy levels gradually increased from acute phases to convalescent phases in ischemic CRVO patients (*$P < 0.05$).

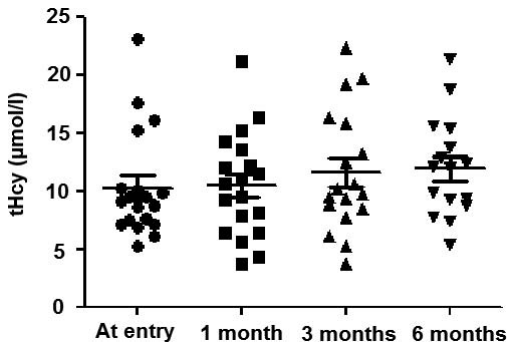

FIGURE 3. Distributions of total plasma homocysteine concentrations in the acute and convalescent phases of nonischemic central retinal vein occlusion. The results show that the tHcy levels gradually increased from acute phases to convalescent phases in nonischemic CRVO patients; however, there were no significant difference ($P > 0.05$).

There were no statistically significant differences in serum folate or vitamin B_{12} levels between CRVO patients and controls at four different time points (Table 3). In addition, no significant difference in the distribution of *MTHFR* C677T mutation genotypes was observed between CRVO patients and controls (Table 4).

DISCUSSION

This is the first long-term follow-up case-control study of CRVO patients with serial measurements of tHcy levels, to the best of our knowledge. The main findings can be summarized as follows. First, the tHcy levels gradually increased from acute to convalescent phases in CRVO patients. Second, sex and age showed a positive correlation with tHcy at all time points in CRVO patients, but there were no correlations between tHcy levels and hypercholesterolemia, hypertriglyceridemia, and smoking. Furthermore, the tHcy levels gradually increased from acute to convalescent phases in ischemic CRVO patients, and at the final follow-up examination, three nonischemic CRVO patients with hyperhomocysteinemia converted to ischemic CRVO.

Homocysteine, a potentially cytotoxic sulfur-containing amino acid, is an intermediate product of methionine metabolism.[13] In the remethylation pathway, homocysteine is remethylated to methionine, which requires vitamin B_{12} as cofactor and methyltetrahydrofolate as a cosubstrate. Thus, hyperhomocysteinemia may be acquired or genetic, as a result of *MTHFR* mutations. Our study showed that there were no statistically significant differences in serum folate and vitamin B_{12} levels between CRVO patients and controls at all four different time points; and there were no statistically significant differences in the distribution of *MTHFR* C677T genotypes in

TABLE 3. Vitamin B_{12} and Folate Levels in CRVO and Controls (Mean ± SD)

	At Entry	1 Mo	3 Mo	6 Mo
Vitamin B_{12}, pg/mL				
CRVO	410 ± 106	416 ± 122	425 ± 119	418 ± 128
Control	421 ± 121	421 ± 121	421 ± 121	421 ± 121
Folate, ng/mL				
CRVO	5.91 ± 2.11	5.86 ± 2.15	5.93 ± 1.95	6.03 ± 2.33
Control	6.12 ± 1.56	6.12 ± 1.56	6.12 ± 1.56	6.12 ± 1.56

Table 4. Genotype Distribution of *MTHFR* C677T Polymorphism in CRVO and Controls [*n* (%)]

	CRVO	Controls	*P**
CC	8 (22.2)	6 (16.7)	0.551
CT	18 (50.0)	23 (63.9)	0.234
TT	10 (27.8)	7 (19.4)	0.405

* Pearson χ^2 test.

CRVO patients and controls. Hence, we speculated hyperhomocysteinemia was associated with the development of CRVO, since the tHcy levels gradually increased from acute phases to convalescent phases in CRVO patients, especially in ischemic CRVO cases. Repair and reorganization of damaged tissue is likely to include methylation of RNA, DNA, and various proteins.[25] Methionine is activated by ATP to produce S-adenosyl methionine (SAM), which donates a methyl group to an acceptor molecule to produce S-adenosylhomocysteine (SAH).[17,26] It has been suggested that an increase in methylation reactions after tissue injury, such as CRVO, results in the conversion of SAM to SAH, which leads to the generation of homocysteine. Hence, the tHcy levels significantly increased from acute to convalescent phases in ischemic CRVO patients compared to nonischemic CRVO, because ischemic CRVO leads to more serious tissue injury.

Previous studies have demonstrated that hyperhomocysteinemia may promote oxidative injury to the vascular bed, which is associated with proliferation of vascular smooth muscle, decreased bioavailability of nitric oxide, expression of acute stress-related genes, altered endothelial function, and enhanced thrombogenicity.[27–29] Several studies have demonstrated elevated tHcy levels to be a potential risk factor in CRVO,[6–13] but others failed to find such an association.[14–20] Consistent with our previous studies, there were no association between tHcy levels in the acute phase after CRVO and CRVO occurrence in a Chinese population.[20] Thus, the role of tHcy in CRVO remains controversial.

There may be several explanations for these differences. First, age is an important confounding factor; there is evidence showing that tHcy increases with age.[30] The present study showed the tHcy levels of the CRVO patients over 60 years old were significantly higher than in patients less than 60 years old at all time points. However, in several studies that demonstrated an association between increased tHcy and CRVO, the patients were significantly older than the control subjects.[6,11,31,32] Second, sex (male or female) is an important confounding factor because homocysteine concentrations are greater in males than in females.[33] Our study showed that the tHcy levels of the female CRVO patients were significantly lower than those in male CRVO patients at all time points. However, in several studies that demonstrated tHcy is an independent risk factor for CRVO, the constituent ratio of male patients was more than that of control subjects.[8,9,12] Third, patient condition (fasting or nonfasting state) is an important confounding factor because homocysteine concentrations are greater in the nonfasting than in the fasting state.[30] Two previous studies demonstrated an association between increased homocysteine and CRVO, but nonfasting blood samples were obtained from patients and control subjects in one study, and nonfasting blood samples were obtained from patients and fasting samples from control subjects in another study.[7,31] Finally, in studies with a retrospective design, patients typically were recruited at variable intervals after onset of CRVO, and the mean time between CRVO occurrence and the determination of tHcy levels ranged from 1 to 69 months, which may have introduced variability in the measured tHcy concentrations. The previous studies demonstrated that the plasma homocysteine concentration gradually increased over time from the acute to the convalescent phase after a stroke,[34] and after acute coronary syndrome.[35] Consistent with the previous studies, the tHcy levels gradually increased from acute to convalescent phases of CRVO in CRVO patients. In addition, the present studies showed no significant differences in tHcy levels between controls and CRVO patients at entry and 1 month after CRVO. However, the tHcy levels of the CRVO patients at 3 and 6 months after CRVO were significantly higher than in controls. Thus, the measurement of tHcy during the convalescent phase after CRVO may have led to the higher tHcy levels in the patients than in the control subjects.

This study has the following limitations: We didn't quantitate the amount of exercise in patients after CRVO. It is known that tHcy levels varied with exercise.[36] We always advised patients to enhance physical exercise and aerobic exercise, because most of them have hypertension or diabetes. However, some patients may have stopped exercising for visual reasons after CRVO. It is difficult to quantitate how many patients follow our advice or how much they reduce exercise owing to visual reasons. In addition, the relationship between tHcy levels and exercise also is being debated. Gaume et al.[37] demonstrated that physical training decreased total plasma homocysteine and cysteine in middle-aged subjects, but Rousseau et al.[38] showed that plasma homocysteine was related to folate intake, but not training status. In addition, our study may be limited by a small sample size. The current study showed that eight patients (22.2%) with CRVO and three controls (8.3%) had hyperhomocysteinemia ($P = 0.101$) at entry. Although the difference is not significant, it seems to be a trend toward association between hyperhomocysteinemia and CRVO occurrence, and may become significant in a larger series. However, there were no significant differences in mean plasma tHcy between controls and CRVO patients at entry ($P = 0.371$). Furthermore, our previous studies included 68 consecutive patients with CRVO and 68 age- and sex-matched controls, and showed no association between tHcy in the acute phase after CRVO and CRVO occurrence in a Chinese population.[20]

It now is widely accepted in clinical practice that ischemic CRVO patients and nonischemic CRVO patients exhibit distinct clinical features, complications, courses, and prognoses, and, therefore, should be managed differently. The present studies showed that at the finally follow-up examination, three patients with nonischemic CRVO converted to ischemic CRVO. Interestingly, all three patients were defined as hyperhomocysteinemia. This suggests that hyperhomocysteinemia may be a predictor of nonischemic CRVO converted to ischemic CRVO, and of a poor prognosis. Further research will be needed to confirm whether reducing plasma tHcy levels in hyperhomocysteinemia patients could prevent the development of ischemic CRVO from nonischemic CRVO, or ultimately contribute to the prognosis of the CRVO patients.

In conclusion, in this work, total plasma homocysteine concentrations were not immediately statistically significantly increased after CRVO, but then they increased in the convalescent period. The mechanism for these results was not attributable to alterations in serum folate, vitamin B_{12} concentrations, and the *MTHFR* C677T genotype, and is not currently and completely explained. It is possible that tHcy increases as a result of the disease process itself. The relationships of tHcy levels and CRVO occurrence remain controversial; thus, further larger multicenter prospective studies may be useful to improve our understanding of the role of the plasma homocysteine level in CRVO.

Acknowledgments

The authors alone are responsible for the content and writing of the paper.

Disclosure: **N. Dong**, None; **B. Xu**, None; **X. Tang**, None

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