

Hyperhomocysteinemia, low vitamin B12, and low folic acid: Are risk factors of cerebral vascular thrombosis in northwest Iran?

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Background: Cerebral vascular thrombosis (CVT) is the thrombosis of intracranial and sinuses. The aim of this is to estimate of risk of low folic acid, low vitamin B12, and hyperhomocysteinemia (hyper-Hcys) for CVT. **Materials and Methods:** A total of 24 patients with CVT and 36 healthy controls participated in a cross-sectional case-control study. The deficient levels of folic acid and vitamin B12 defined as <10th percentile of folic acid and vitamin B12 level and hyper-Hcys was defined as >90th percentile of homocysteine of control group. **Results:** Patients had higher levels of total homocysteine (tHcys) than controls (14.7 ± 6.5 vs. 6.4 ± 2.7 $\mu\text{mol/L}$, $P = 0.001$). Also, vitamin B12 level in case group was lower compared to control subjects (185.4 ± 58 vs. 299 ± 75 ng/mL , $P = 0.001$). Hyper-Hcys and low vitamin B12 were significantly more prevalent in CVT patients than controls. Although, significant independent association with risk of CVT was found for hyper-Hcys [adjusted odds ratio (OR) 14.3, 95% confidence interval (CI): 2.6-77.1, $P = 0.002$] and low vitamin B12 (adjusted OR 24.6, 95% CI: 2.3-262.9, $P = 0.008$). Association between low folic acid and risk of CVT was not significant. A significant negative correlation was found between the levels of tHcys and vitamin B12 ($r = -0.32$, $P = 0.01$). **Conclusion:** Hyper-Hcys and low vitamin B12 were related with the high risk for CVT.

Key words: Cerebral vascular thrombosis (CVT), deficiency, folic acid, hyperhomocysteinemia (hyper-Hcys), vitamin B12

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INTRODUCTION

Cerebral vascular thrombosis (CVT), a type of cerebrovascular disease, is the thrombosis of intracranial and sinuses.^[1] For the first time, CVT was recognized at the early nineteenth century and that time thought CVT was infective disease resulting in seizures and bilateral or other focal abnormalities.^[2] Today, CVT is recognized as a noninfective disorder accompanied by various clinical symptoms and mortality less than 10%.^[2] The approved diagnostic methods for CVT are magnetic resonance imaging (MRI) and magnetic resonance angiography.^[3] In addition, heparin is the first line of treatment. Because of wide spectrum and symptoms and cases, diagnosis of CVT is still overlooked and delayed.^[3]

Several causes have been considered in the etiology of CVT. These factors including all causes of deep vein thrombosis in the legs, genetic, malignancy, prothrombin related disorders, deficiency of folic acid and vitamin B12, and hyperhomocysteinemia (hyper-Hcys).^[3,4]

Hyper-Hcys or rising of homocysteine in blood has been related to smoking, old age, renal and liver diseases, and vitamins deficiency as well as genetic disorders affecting the remethylation pathway of homocysteine metabolism. High level of total homocysteine results from interaction of genetic and related determinants. Moreover, deficiencies of folic acid and vitamin B12 are the other causes of hyper-Hcys.^[5] In fact, lowering effect of vitamin B12 and folic acid on homocysteine has been seen in clinical trials.^[6,7]

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The association of high levels of homocysteine with vascular damages such as thrombosis, atherosclerosis, and some ischemic diseases as well as other neurological disease such as migraine has been found in several investigations.^[8,9] Hyper-Hcys has been considered as a risk factor for CVT in several studies.^[10,11] However, no clear information is available for relation between hyper-Hcys and related vitamins deficiency with CVT in Iranian patients.

Some evidences have evaluated the association of CVT with vitamin B12 and folic acid deficiency as well as hyper-Hcys.^[12,13] Vitamin supplementation with folic acid and cobalamin lowers the plasma levels of tHcy in most cases.^[6,7] Thus, hyper-Hcys as a risk factor for CVT may relate to folic acid and vitamin B12 deficiency. To the our knowledge, there is no local study investigating nutritional deficiency of vitamin B12 and folic acid with CVT in northwest Iran. We conducted this study to compare vitamin B12 and folic acid deficiency prevalence between CVT patients and healthy subjects and to evaluate the relationship of hyper-Hcys, low vitamin B12, and low folic acid with the risk of CVT occurring in northwest Iran.

MATERIALS AND METHODS

Study design and participants

A total of 24 patients with CVT and 36 healthy controls participated in a cross-sectional case-control study from May 2010 to March 2012. The patients with definite diagnosis of CVT referred to Emam Reza Hospital of Tabriz were invited to take part in the study. CVT was diagnosed by computed tomography (CT) or MRI. The patients with conditions that influenced homocysteine concentration, such as renal or thyroid disease, or who were in anticonvulsant therapy with phenytoin or carbamazepine were excluded from study. The control participants were chosen from partners with no cancer, liver disease, renal failure, and surgery who agreed to take part in study matched for age (± 5 years) with the patients with cerebral vein thrombosis. They were recruited at the same time as cases. The case subjects ranged from 14 to 61 years old (mean = 32.2, SD = 10.8) who were in two gender (men; $N = 4$, women; $N = 20$). The control participants (men; $N = 9$, women; $N = 27$) ranged from 14 to 61 years old (mean = 33.04, SD = 11.6). The presence of circumstantial thrombosis risk factors, such as oral contraceptive drugs intake, pregnancy, cancer, surgery, and trauma, were reported for both patients and controls. This study was approved by Ethics Committee of the Tabriz University of Medical Sciences. All of the participants were informed about the design of the study and were assured about data confidentiality, safeness of the study, and the voluntary nature of their participation. All the participants provided written informed consent.

Procedures and variables assessment

Blood sampling was done at the breakfast and after overnight fasting and the samples were stored at -80°C until analyzed. Serum total homocysteine concentrations were determined on frozen samples by high-performance liquid chromatography (HPLC) method (KNAUER, Germany), coupled with fluorescence detector.^[14] For evaluating folic acid and vitamin B12, the samples were collected in tubes protected from light and levels of folate and vitamin B12 were measured simultaneously in the frozen serum aliquot by a double labeled radioassay kit (ICN Pharmaceuticals, New York) (Stat Fax 4200, Awareness Technology, USA) in all cases and controls.

Statistical analysis

Statistical analysis was conducted using SPSS 15 for Windows (SPSS, Chicago, IL). Variables were tested for normal distribution using the Kolmogorov–Smirnov test. For normally distributed continuous variables, mean and standard deviation were calculated, and for nonnormally distributed variables, median and range were used. The χ^2 test was used for categorical variables. Mann–Whitney U -test was used for comparing homocysteine level between groups because data were not normally distributed. In the patient's group, vitamin B12 levels in two samples were observed as outlier. Therefore, the final analysis was done on 22 participants in the case group. We used independent t -test for comparing serum levels of folic acid and vitamin B12 between the case and control groups. The deficient level of folic acid and vitamin B12 defined as $<10^{\text{th}}$ percentile of the control group.^[12] Similarly, hyper-Hcys was defined as $>90^{\text{th}}$ percentile of homocysteine level of the control group.^[12] We used crude odds ratio (OR) and 95% confidence interval (CI 95%) with by simple cross-tabulation for estimating the risk of hyper-Hcy, vitamin B12, and folate deficiency for CVT. Adjusted OR for CVT was obtained by multiple logistic regression analysis. The significance level was established at a value of $P = 0.05$.

RESULTS

General characteristics and clinical outcomes

The baseline descriptive characteristics of patients and controls are shown in Table 1. There was no significant difference in age between patients and controls. Four patients (16.7%) and 12 controls (33.3%) were male and no significant gender proportion difference was found between groups. The most common symptoms of patients were headache (83.3%), focal symptoms (41.7%), seizure (33.3%), and nausea and vomiting (29.6%). Results of imaging showed that the sagittal sinuses in 13 patients (54.2%) and lateral sinuses in 8 patients (33.3%) are the most typically involved sinuses.

Levels of tHcys, vitamin B12, and folic acid and related correlation

The 90th percentile for tHcys was 10.2 $\mu\text{mol/L}$ and 10th percentile for folic acid and B12 levels in the control group were 211.9 ng/mL and 11.69 ng/mL, respectively. These values were defined as cutoff levels for hyper-Hcys, low folic acid, and low vitamin B12. Although patients had higher levels of tHcys (14.7 ± 6.5 vs. 6.4 ± 2.7 $\mu\text{mol/L}$, $P = 0.001$) and lower levels of vitamin B12 (185.4 ± 58 vs. 299 ± 75 ng/ml, $P = 0.001$) than controls, folic acid did not differ between groups. Hyper-Hcys and low vitamin B12 were significantly more prevalent in CVT patients than that in controls (70.7% vs. 7.9%, $P = 0.001$; 58.3% vs. 2.6%, $P = 0.001$) [Table 2]. Although a significant negative correlation was found between levels of tHcys and vitamin B12 ($r = -0.32$, $P = 0.01$), no significant association was observed between tHcys and folic acid neither between vitamin B12 and folic acid levels (data were not shown).

Risk assessment

Crude and adjusted ORs are presented in Table 2. Adjusted OR was estimated by adjusting each variable for the others. After adjusting for low vitamin B12 and low folic acid, hyper-Hcys showed significant independent association with risk of CVT (adjusted OR 13.5, 95% CI: 2.5-72.5, $P = 0.002$). In addition, low vitamin B12 remained a potent risk factor for CVT after adjusting for low folic acid and hyper-Hcys (adjusted OR 3.2, 95% CI:1.8-34.5, $P = 0.015$).

Table 1: Baseline characteristics and laboratory findings of the study

Variable ^a	Patients (N = 22)	Controls (N = 36)	P
Age (years)	32.2 \pm 10.8	35.3 \pm 11.9	0.3 ^b
Gender: N (%)			0.07 ^c
Male	4 (16.7)	12 (33.3)	
Female	20 (83.3)	24 (63.2)	
Plasma Hcys ^d ($\mu\text{mol/L}$)	15 (3.9-27.4)	5.95 (1-14.3)	0.001 ^e
Plasma vitamin B12 (ng/mL)	185.4 \pm 58	299 \pm 75	0.001 ^b
Plasma folic acid (ng/mL)	16.5 \pm 9.4	14.8 \pm 2.6	0.4 ^b

^aValues expressed as mean \pm SD; ^bIndependent t-test ^c χ^2 test; ^dValues expressed as median (range); ^eMann-Whitney U-test

DISCUSSION

Although studies have reported that hyper-Hcys is an important risk factor for CVT, there are rare evidence in Iranian CVT patients. In our study, we evaluated homocysteine and related vitamins in the CVT patients and compared with controls. Our study showed that CVT patients had higher levels of homocysteine and lower level of B12 compared to controls. There was no significant difference in folic acid between case and control groups. In agreement to our study, a study by Martinella *et al.*^[15] showed that the CVT patients had higher level of homocysteine than the controls, but the levels of B12 and folic acid did not differ between the groups. In line with our results, a study conducted in Indian CVT subjects revealed a higher level of homocysteine, lower level of folic acid, and B12 compared to control volunteers.^[13] Similarly, another study carried out among Mexican CVT patients showed higher level of Hcys and lower level of folic acid in the CVT group than controls.^[12] The small sample size as a potent limitation in our study as a potent limitation and various dietary pattern as well as different value of animal protein intake, as a main source of vitamin B12 and folic acid in diet, may be explanations for disparity between studies.

A significant reverse correlation was revealed between B12 levels and tHcys. But this correlation was not found between folic acid level and tHcys. In contrast, Nageraja *et al.* reported significant correlation between tHcys and folic acid levels in the CVT patients but not between tHcys and vitamin B12 levels.^[13] Similar results were found in other studies conducted in CVT and coronary artery disease.^[16,17] In other study, Abdulle *et al.* found that total plasma homocysteine concentration had an inverse correlation with both vitamin B₁₂ and folate.^[18] In agreement with our study, the results of study conducted in Iranian population showed significant negative correlation between vitamin B12 and homocysteine concentration.^[19] It is implied that in our study subjects, deficiency of vitamin B12 may be a cause for the increase in fasting Hcys.

Since 1998, the fortification of cereal grain flour products with folic acid was mandated in the United States. Supplementation

Table 2: Risk estimation of hyperhomocysteinemia, low vitamin B12, and low folic acid for CVT

Variable ^a	Patients (N = 22)	Controls (N = 36)	Crude OR (95% CI)	P	Adjusted OR ^c (95% CI)	P
Hyper-Hcys (>10.2 $\mu\text{mol/L}$)	15 (68.2)	3 (7.9)	28.3 (6.5-123.1)	0.001	14.3 (2.6-77.1)	0.002
Non-hyper-Hcys (\leq 10.2 $\mu\text{mol/L}$) ^b	7 (31.8)	33 (92.1)	1		1	
Low vitamin B12 (<211.9 ng/mL)	14 (62.7)	1 (2.6)	51.1 (6.1-442.6)	0.001	24.6 (2.3-262.9)	0.008
High vitamin B12 (\geq 211.9 ng/mL) ^b	8 (36.3)	35 (97.4)	1		1	
Low folic acid (<11.69 ng/mL)	6 (27.2)	3 (7.9)	3.5 (0.4-31.9)	0.056	3.5 (0.4-31.9)	0.25
High folic acid (\geq 11.69 ng/mL) ^b	16 (72.8)	33 (92.1)	1		1	

Hyper-Hcys = Hyperhomocysteinemia; ^a Values expressed as N (%); ^b Considered as reference group in logistic regression analysis; ^c Hyper-Hcy was adjusted for low vitamin B₁₂ and folate. Low vitamin B₁₂ and folate were adjusted for each other and for hyper-Hcy

with folate resulted in about 20-25% reduction in tHcy levels and cerebrovascular diseases in the United States. The government of Iran passed a law in 2005 to require wheat flour fortification, and the law was implemented nationwide by 2007. In northwest Iran, the Tabriz Registry of Congenital Anomalies reported a NTD rate of 16.9 per 10,000 live births in 2005 which dropped to 7.0 per 10,000 in 2006.^[19]

To the our knowledge, this study is a first evaluation of folic acid and B12 levels in the patients with cerebral vein thrombosis in northwest Iran after flour fortification with folic acid program. Results of our study indicate that folic acid fortification may cover folic acid deficiency as a risk factor for hyper-Hcys.

It seems that folic acid fortification program among Iranian population may lead to increase of folic acid intake and no significant association between folic acid and tHcys was found in CVT patients.

Findings of our study suggested that Hyper-hcys may be a potent risk factor for CVT patients. In our study, the OR for CVT in people with hyper-Hcys was 14.3 after adjusting for low vitamin B12 and folic acid levels. The study by Nagarja *et al.*^[13] showed that adjusted OR for puerperal CVT in patients with hyper-Hcys was 10.8 (95% CI, 4.0-29.4; $P < 0.001$). Similarly, Cantu *et al.*^[12] found an OR of 3.9 for CVT in subjects with hyper-Hcys. In consistent, the case control study by Martinelli *et al.*^[15] found hyper-Hcys in 33 of 121 patients (27%) and 20 of 242 healthy controls (8%; OR, 4.2; 95% CI, 2.3 to 7.5).

Our study found that low vitamin B12 not low folic acid had a significant independent association with the risk of CVT. This suggests that risk of CVT is mediated through hyper-Hcys and vitamin B12 deficiency. The study by Cantu *et al.* in Mexican people found that hyper-Hcys as well as low level of folic acid and vitamin B12 had independent association with the risk of CVT, suggesting mechanisms other than hyper-Hcys should be considered for the risk of CVT.

Our study is a case control study and therefore it has some limitations. Confounding variables were not eliminated and controls were chosen from the same source of cases. Level of tHcys is dependent on renal function. Although we did not measure creatinine levels, subjects were selected from the people reporting no renal disease. In our study, because of sample size limitation we found wide rages of CI compared to other studies. In addition, we did not record a history of diet.

CONCLUSION

In conclusion, our findings are in agreement with the hypothesis that hyper-Hcys is associated with the increased risk of CVT. Furthermore, low vitamin B12 level was related

significantly with high risk for CVT, but folic acid level was not associated directly with risk of CVT. Further, long-time studies with large sample size are needed to assess the mechanism and effect of hyper-Hcys, low vitamin B12, and low folic acid on the risk of CVT.

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Conflicts of interest

There are no conflicts of interest.

AUTHOR'S CONTRIBUTION

AT contributed in the conception of the work, conducting the study, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work.

ND contributed in the conception of the work, data collecting as independent role, conducting the study, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work.

M Kh contributed in the conception of the work, writing original manuscript as independent role, conducting the study, data analyzing, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work.

MS contributed in the conception of the work, conducting the study, revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work.

SSA contributed in the conception of the work, analyzing of data as independent role, revising the draft, and agreed for all aspects of the work.

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