Hyperhomocysteinemia as a Risk Factor for Saccular Intracranial Aneurysm: A Cohort Study in a Chinese Han Population

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Background: We evaluated the possible relationships between serum total homocysteine and folate and Vitamin B_{12} in patients with intracranial aneurysm. *Methods:* We enrolled consecutive patients with intracranial aneurysm from the Han population who were admitted to the hospital, as well as control subjects who received medical examination on an outpatient basis. The serum total homocysteine, folate, and Vitamin B_{12} levels were measured in patients with intracranial aneurysm and the control group, and the associations between those factors were analyzed using multivariate logistic analysis. *Results:* A total of 140 patients with intracranial aneurysm and 140 control subjects were enrolled from July 2014 to December 2015. The mean serum total homocysteine level in the patient group (19.98 ± 10.84 μmol/L) was significantly higher than that in the control group (15.13 ± 5.55 μmol/L, $P < .001$). The serum total homocysteine level was negatively correlated with folate and Vitamin B_{12} levels ($r = -.349$, $P < .001$; $r = -.531$, $P < .001$, respectively) in the patient group. Homocysteine had an adjusted odds ratio of 2.196 (95% confidence interval: 1.188-4.057, $P = .012$) for the development of intracranial aneurysm. *Conclusions:* The present study provides evidence regarding the association between serum total homocysteine and folate and Vitamin B_{12} in patients with intracranial aneurysm. Hyperhomocysteinemia is an independent risk factor for intracranial aneurysm, and folate and Vitamin B_{12} are protective against intracranial aneurysm due to their roles in regulating the metabolism of homocysteine. **Key Words:** Intracranial aneurysm—homocysteine—Vitamin B_{12}—folate—folic acid—hyperhomocysteinemia—risk factor.

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Introduction

Subarachnoid hemorrhage (SAH) resulting from intracranial aneurysm (IA) rupture is associated with significant morbidity and mortality.[1,2] The prevalence of unruptured IAs in the general population is about 3%.[3] SAH accounts for approximately 5% of all strokes and occurs at a relatively young age compared with other stroke subtypes.[4] There is great interest in the prevention of IAs, including discussions preventing IA formation and avoiding catastrophic sequelae. Although a rare familial form has been described in the literature, IAs are generally considered to be the result of acquired vascular damage, such as that caused by smoking, hypertension, and other traditional risk factors. However, causal associations and the mechanisms by which IAs form, enlarge, and rupture remain poorly understood.[5,6]

Previous studies have found that hyperhomocysteinemia (HHcy) is an independent risk factor for cerebrovascular disease[7] that can lead to damaged endothelial cells, degraded extracellular matrix, smooth muscle cell apoptosis, and the infiltration of inflammatory cells, among others.[8-10] These features overlap what is known about the pathogenic mechanism of IA.[11-14] In addition, HHcy might be a risk factor for stroke,[9] abdominal aortic aneurysms,[15,16] and cervical arterial dissection.[17]

HHcy has been reported to impair endothelium-dependent dilatation of the middle cerebral artery in humans.[10] Tests in animals, although limited, have demonstrated that methionine-rich, diet-induced HHcy accelerates the formation of cerebral aneurysms in rats, potentially through differential effects on the expression of molecules critical for vascular wall modeling.[18] Elevated homocysteine (Hcy) levels have been associated with deficient or low availability of folate and Vitamin B_{12} ($VitB_{12}$),[19,20] suggesting that controlling HHcy using an intervention based on folate and $VitB_{12}$ may be possible, thereby preventing the formation of IAs and reducing their incidence.

In this study, the levels of serum total homocysteine (tHcy), folate, and $VitB_{12}$ were measured in patients with IAs. The aims of this study were to evaluate the possible relationship between Hcy and IA, and to examine the associations between folate and $VitB_{12}$ and serum tHcy in patients with IAs.

Subjects and Methods

Study Design and Inclusion and Exclusion Criteria

The subjects of this study were consecutive patients with IA of Han nationality admitted to the Department of Neurosurgery at the People's Hospital of Shanxi Province and who underwent brain computed tomography (CT) or magnetic resonance imaging and cerebral angiography (completed within 48 hours of onset). Between July 2014 and December 2015, patients with a ruptured IA (accompanied by SAH or ventricular hemorrhage) or unruptured IA were treated with surgery or endovascular treatment. Control subjects (medical examination outpatients) were recruited from our hospital and were determined to be free of overt acute disease using a questionnaire.

Patients and control subjects were included if they were between 18 and 80 years old, whereas patients were excluded if they underwent surgery >72 hours after rupture. The following were the exclusion criteria for patients and control subjects: malignant tumor, chronic insufficiency of the liver or kidney function, hematologic diseases, peripheral vasculitis, dyspepsia, malnutrition, hypothyroidism, pregnancy, recent (within 3 months) surgical history, drug abuse, and oral vitamin, folate, or antiepileptic medications. Throughout this study, the term "aneurysm" refers to saccular IAs, unless otherwise specified. All relevant information regarding cerebrovascular angiography was reviewed by 2 local neurologists.

We assessed the characteristics of all study subjects in both the IA and control groups, including cigarette smoking, present alcohol use, family history of aneurysm, hypertension (including systolic blood pressure and diastolic blood pressure), diabetes, hypercholesterolemia, aspirin use before admission, antihypertensive treatment, and history of atherothrombotic events. Age significantly differed between the IA and control groups. The clinical indices of IA assessed included the Hunt and Hess Scale, CT-Fisher grade, and aneurysm location, quantity, and diameter (Table 1). All patients or their relatives provided informed consent, and the study protocol was approved by the ethics committee of our institution.

Serum Sample Collection

The serum samples of the patients were collected on the day of hospital admission. Venous blood samples were collected in empty 4-mL tubes in the fasting state, and centrifuged within 30 minutes at 3500 *g* for 15 minutes at 4°C. Serum was separated from the blood samples within 1 hour, aliquoted into a plastic tube, and frozen at −70°C until analyzed.

Measurement of Hcy, Folate, and B_{12} in Serum

Serum Hcy concentration was measured using an enzymatic cycling assay (Beckman Coulter AU5800, Chaska, MN). The serum levels of folate and $VitB_{12}$ were measured using a chemiluminescent microparticle immunoassay (Beckman Coulter DXI800, Tokyo, Japan). Biochemical parameters were analyzed at the Shanxi Centers for Clinical Laboratory. All experiments were performed in accordance with relevant guidelines and regulations. For interpretation, tHcy concentration was dichotomized into HHcy (≥15 μmol/L) and normal tHcy level (<15 μmol/L) based on the laboratory reference range. For these assays, the normal serum values of folate and $VitB_{12}$ were ≥4 μg/L