Hyperhomocysteinemia in ulcerative colitis is related to folate levels

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INTRODUCTION

The risk for thromboembolic complications is increased in patients with inflammatory bowel disease (IBD). The incidence of arterial and venous thromboembolic disease in patients with ulcerative colitis (UC) and Crohn’s disease (CD) has been reported between 1% and 8%[1-4], rising to an incidence of 39% in some autopsy studies[5]. Several studies have shown that a hypercoagulable state involving all components of clotting system exists in IBD[6-8]. This hypercoagulable state may be related to an increased tendency for thromboembolic events and may be linked to the disease pathogenesis through promoting microthrombi formation in intestinal microcirculation[7-8]. The etiology and pathogenesis of the hypercoagulable state in IBD have not been fully elucidated but may be induced through a procoagulant effect of proinflammatory cytokines[9-13] in combination with acquired or genetic defects of clotting factors (protein S, protein C, antithrombin, factor V Leiden, prothrombin mutation 20210A, and antiphospholipid antibodies)[14-16]. Recently, the factor V Leiden has been implicated in the increased risk of venous thrombosis in IBD patients[17-19].

Homocysteine (Hcys) is a non-essential, sulfur-containing amino acid formed during the metabolism of methionine. Mild hyperhomocysteinemia (hHcys), which occurs in approximately 5-7% of the general population, has been proved to be thrombogenic and an independent risk factor for coronary artery disease[20], arterial and venous thrombosis[21-26]. Elevated levels of Hcys may result from abnormalities in metabolism pathways due to inherited abnormalities of the enzymes involved or nutrient deficiencies such as insufficient of folate and vitamins B1, B12, B6, and B15[27,28].

The mechanism by which hHcys promotes thrombosis is uncertain, but it may be related to a hypercoagulatory state due to endothelial dysfunction[29-30].

Vitamin B12 and folate deficiency are relatively common conditions in IBD (especially in active disease) through malnutrition, malabsorption or antifolate drugs such as methotrexate and sulfasalazine (SASP). Deficiencies of key nutrients/cofactors in Hcys metabolism pathways (B1, B12, B6, and folate) might lead to raised Hcys levels in IBD. The association between IBD and hHcys has been shown in patients with IBD[17-19], rising to an incidence of 39% in some autopsy studies[5]. Several studies have shown that a hypercoagulable state involving all components of clotting system exists in IBD[6-8]. This hypercoagulable state may be related to an increased tendency for thromboembolic events and may be linked to the disease pathogenesis through promoting microthrombi formation in intestinal microcirculation[7-8]. The etiology and pathogenesis of the hypercoagulable state in IBD have not been fully elucidated but may be induced through a procoagulant effect of proinflammatory cytokines[9-13] in combination with acquired or genetic defects of clotting factors (protein S, protein C, antithrombin, factor V Leiden, prothrombin mutation 20210A, and antiphospholipid antibodies)[14-16]. Recently, the factor V Leiden has been implicated in the increased risk of venous thrombosis in IBD patients[17-19].

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in some recent studies, reporting an increased prevalence of hHcys in IBD (both UC and CD)\(^{31-36}\).

The aim of this study was to evaluate whether Hcys levels were elevated compared to a group of healthy controls and whether Hcys levels were related to vitamin B\(_{12}\) and folate serum concentrations, disease activity, disease extent or history of thrombotic complications.

**MATERIALS AND METHODS**

**Patients and control population**

Forty patients with UC (20 females and 20 males, mean age 41.65±15.21 years, range 17-71 years) were consecutively recruited from our outpatient clinic between February 1999 and February 2000. Diagnosis of UC was based on standard clinical, endoscopic and histological criteria. A detailed clinical history was taken from each patient regarding current symptoms, activity and duration of disease, extraintestinal manifestations, present medication, smoking status, and thrombotic complications. Patients with significant liver or kidney diseases were excluded. Endoscopy was performed in all patients in order to evaluate endoscopic activity, disease extension and histological confirmation and grading. Blood samples from fasting UC patients were collected. Serum Hcys, folate and vitamin B\(_{12}\) levels were determined.

Serum Hcys levels were also measured in blood samples from 50 healthy control subjects (HC) with similar age and gender (25 females and 25 males, age 39.96±14.33 years, range 17-72 years), under similar conditions and in the same laboratory. Healthy control subjects were visitors in the outpatient clinic of Hematology Department and had no known diseases, or any clinical or laboratory evidence of metabolic, neoplastic or inflammatory disease. They also had no history of thromboembolic disease. Patients and healthy controls were from the same geographical area (Northern Greece) and had Greek ancestry. Patients and controls reported that they had no daily alcohol intake above 35 g, and no use of drugs affecting Hcys status (phenytoin, theophylline, and vitamin supplements).

**Measurements**

Total serum Hcys concentrations were measured by the IMx homocysteine assay, which is a fluorescence polarization immunoassay (FPIA, Abbott Laboratories). Reference serum Hcys concentrations for both men and women were <15 μmol/L. Vitamin B\(_{12}\) and folate serum levels were measured by enzymatic immunoassays (ELISA). Reference ranges for vitamin B\(_{12}\) and serum folate were >223 pg/mL and >2.8 ng/mL respectively.

**Statistical analysis**

Descriptive statistics for continuous variables (including means and medians) were calculated. Categorical variables were described using proportions. Odds ratios and the 95%CI for the risk of hHcys in UC patients, as compared to healthy controls, were calculated. Multivariate logistic regression was used to adjust these crude odds for confounding differences in sex. Comparisons of continuous variables between the two groups were made by Student’s t-test for normally distributed data or by Mann-Whitney U-test when data were not normally distributed. Fisher’s exact test was used for the comparison of proportions. Pearson’s correlation coefficient was calculated to describe the relationship between variables. Multiple linear regression analysis was performed in order to study the influence of individual factors on Hcys levels (treated as continuous variable). Since the concentrations of Hcys, folate and B\(_{12}\) were not normally distributed, correlation and regression analysis were performed with log-transformed data. Statistical analysis was performed using the SPSS for Windows package (version 11.0, SPSS, Chicago, IL, USA).

**RESULTS**

**Demographics**

The baseline characteristics of the patients and controls are shown in Table 1. There were no significant differences in age between healthy controls and patients, or between sexes or between study groups. There were also no significant differences in duration of disease, disease activity, endoscopic score, disease extent, smoking, and use of medication between sexes in UC group.

**Table 1 Epidemiological and clinical data of study subjects**

| Subjects (n) | UC patients (95%CI) | Healthy controls (95%CI) |
|-------------|---------------------|--------------------------|
| Gender (female/male) | 20/20 | 25/25 |
| Mean age (yr±SD)(range) | 41.65±15.21 [36.78–46.52] | 39.96±14.33 [35.89–44.03] |
| Mean disease duration (mo±SD)(range) | 56.6±63.18 [36.44–76.86] | 3 (3–216) |
| Current smoking (%) | 9 (22.5) |
| Extent of UC (%) | Rectum/sigmoid 9 (22.5) | Left colitis 22 (55.5) |
| Pancolitis | 9 (22.5) |
| Activity of UC (%) | Active 28 (70) | Inactive 12 (30) |
| Extraintestinal complications (%) | 6 (15) |
| Thrombotic complications (%) | 2 (5) |
| Medical treatment | None 1 (2.5) | 5-ASA 35 (87.5) |
| SASP | 1 (2.5) |
| Steroids | 17 (42.5) |
| Immunosuppressors (AZA/6-MP) | 11 (27.5) |

**Homocysteine determination and associations**

The median serum levels of Hcys in UC patients were similar to those in controls (12.26 μmol/L [range 7.15-35.8 μmol/L] \(vs\) 12.32 μmol/L [range 5.97-22.06 μmol/L], \(P = 0.518\), but hHcys was more prevalent in UC patients (30% [12/40] \(vs\) 10% [5/50], \(P = 0.028\)) than in controls. Male sex had higher median serum levels of Hcys both in HC and in UC groups (Table 2). Logistic regression analysis showed an odds ratio of 3.857 [95%CI: 1.22-12.12] for hHcys in the UC group as compared to healthy controls. Advanced age and
male sex were associated with higher Hcys levels, and there was not any difference in age and sex, but males had significant higher Hcys values in both study groups and we believe that sex might be a significant confounder. The adjusted odds ratio for the sex difference was 4.125 (95% CI: 1.26-13.44).

**Table 2 Serum Hcys levels in male and female subjects in study groups**

| Heys (µmol/L) | Male          | Female          | p          |
|--------------|---------------|-----------------|------------|
| Controls     | 13.7 (8.17–22.06) | 11.94 (5.97–16.99) | 0.005      |
| UC           | 14 (9.13–35.8) | 11.07 (7.15–26.6) | 0.011      |
| Total        | 13.72 (8.17-35.8) | 11.87 (5.97–26.6) | 0.0003     |

Values are expressed as medians (range). Comparisons were performed using Mann-Whitney’s test.

**Serum homocysteine, folic acid and vitamin B12 levels in ulcerative colitis patients**

In UC patients, the median serum levels of folate and vitamin B12 were 7.2 ng/mL (range 2.4-13.4 ng/mL) and 431 pg/mL (range 195-1,430 pg/mL) respectively. One patient had serum folate below the lower limit (2.4 ng/mL) and two others had normal folate near the lower limit (2.9 and 3.4 ng/mL respectively). All three had high levels of Hcys (the three highest values). One patient had vitamin B12 below the lower limit (195 pg/mL) and two others had normal B12 near the lower limit (226 and 237 pg/mL, respectively). The patient with low B12 and one of the two others had high levels of Hcys. These patients had no overlapping low values for both folate and B12 levels. The disease duration, activity, extent, endoscopic severity, medical treatment, and smoking status did not significantly influence Hcys, folate, and vitamin B12 levels. There were no significant differences in levels of folate and vitamin B12 between sexes.

**Predictors of hyperhomocysteinemia in ulcerative colitis**

Because the concentrations of Hcys, folate and B12 were not normally distributed, correlation and regression analysis were performed with log-transformed data. To directly assess the effect of age, folic acid and B12 on Hcys levels, the Pearson correlation coefficients between these variables were determined, as shown in Table 3. A relatively strong inverse correlation was observed between log-transformed serum levels of Hcys and log-transformed serum concentrations of folate (r = -0.466, P = 0.002). To further define the role of folic acid and B12 in determining Hcys levels, a multiple regression analysis was performed where log-Hcys was the dependent variable whereas age, gender, log-B12, log-folic acid, smoking and history of thrombosis were the independent variables. Multiple linear regressions revealed the following variables to be significant independent predictors of Hcys levels in UC patients: male sex, folate, and B12 deficiency or lower serum values (r² = 0.4; P<0.001).

**Homocysteine metabolism in ulcerative colitis patients with previous thrombotic events**

Two patients had a history of previous thrombotic events. hHcys was found in one of them. This patient had folate deficiency and the highest value of Hcys. The other one had vitamin B12 close to lower normal limit without hHcys.

**DISCUSSION**

This study showed that although Hcys levels were similar in UC patients and healthy control subjects, hHcys (serum Hcys ≥ 15 µmol/L) was more common in UC patients than in healthy controls (adjusted odds ratio, 4.125; prevalence 30% vs 10%). Increased Hcys levels and high prevalence of hHcys in IBD patients have been reported in previous studies[31-36]. In our study, similar findings were observed in this cohort of UC patients with a slightly higher prevalence of hHcys (30%) than in other studies (10-26%)[31,32,35,36].

Our analysis showed that male sex and low serum levels of folic acid and vitamin B12 were correlated with high Hcys levels. Multiple regression analysis and Pearson’s correlation coefficient showed that serum folic acid was the most significant predictor of Hcys levels. We did not find significant correlation between age, disease activity, medication, smoking status, and Hcys levels in this UC cohort. Chowers et al.[33], have observed similar findings in a group of patients with CD.

We did not study the frequency of MTHFR C677T variant in our study groups. In a previous study, Mahmud et al.[31], reported that the frequency of the homozygous C677T mutation, which results in slower synthesis of 5-methyltetrahydrofolate, is increased from 7.3% in controls to 17.5% in patients with UC and is related with high Hcys levels especially in folate deficiency status. However, in that study, Hcys levels were also elevated in patients with IBD, with no mutation to MTHFR enzyme. The levels of Hcys decreased after folate supplementation, regardless of the fact that the genotype of the mutation was detected or not. It has been suggested that folate status should be addressed in all IBD patients and prophylactic folate supplementation has been recommended to all patients with IBD. In our study, the prevalence of hHcys in UC patients was much higher than the frequency of homozygous mutation of MTHFR as detected by Mahmud et al.[31]. The differences between prevalences of hHcys and MTHFR mutant can be explained by the existence of additional factors affecting Hcys levels. Vitamin deficiencies can be a significant
contributing factor as it has been supported by our study and previous reports. The suboptimal vitamin status in IBD patients can be due to a combination of several factors. Folate levels may be low due to either inadequate dietary intake, or increased utilization, or drug effects, mainly SASP. In our study, one patient was receiving SASP and had low folate levels and hHcys. Other studies have conflicting conclusions about the significance of SASP antifolate effects. Disease activity may contribute to increased demand for folate due to inflammation, but like Chowers et al., we did not find any correlation between disease activity and folate or hHcys levels. Furthermore, Chowers et al., found no change in Hcys levels, despite a significant improvement in the disease activity in a group of CD patients. These data point out that an inadequate intake of folate may be the most significant factor affecting folate levels in IBD patients.

The prevalence of thromboembolic complications in IBD patients is higher than that in the normal population, in large retrospective studies 1.3-8% of the patients develop these complications. The pathogenesis of thromboembolism in IBD is unknown, but it seems to be multifactorial. In our study we found a high prevalence (2/40; 5%) of thromboembolic events in this group of UC patients. hHcys was found in one of them. There are case reports that hHcys was noted in the test results in IBD patients with thrombosis. In our study a correlation between hHcys and history of thrombosis was noted, but the number of patients is too small to supply safe conclusions. Nevertheless high Hcys levels may predispose IBD patients to thrombotic complications in combination with other existing circumanstual or permanent risk factors.

In conclusion, UC patients have a higher prevalence of hHcys than healthy controls. hHcys in these patients is related to low folate and B12 status but not to disease activity. All UC patients, irrespective of disease activity, are at risk for vitamin deficiencies. It is recommended that all patients should have an assessment of the nutritional status in order for the vitamin deficiencies to be detected and that they should also receive folate and vitamin B complex supplements for protection from complications of Hcys.

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