Hypercoagulable state in idiopathic ulcerative colitis: role of hyperhomocysteinemia and hyperfibrinogenemia

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Background: Previous reports on hypercoagulable factors in inflammatory bowel diseases involve heterogeneous populations and patients on various medications.

Aims: To determine the frequency of thrombotic complications in ulcerative colitis (UC); to evaluate for hyperhomocysteinemia and its relationship to vitamin B12 and folate levels and methylene tetrahydrofolate reductase (MTHFR) mutation; and to evaluate for hyperfibrinogenemia and factor V Leiden mutation.

Methods: Eighty-six adult patients with UC were seen during the study period; 28 of them underwent blood tests and constituted the study population. Patients who received medications that affect these factors were among the 58 excluded. Tests were obtained at baseline and after 2 months during remission. Patients received folic acid in addition to treatment for UC.

Results: Vascular thrombotic events were noted in 4 patients during follow up. Hyperhomocysteinemia was detected in 11 (39.3%) patients (controls 15/100, p=0.007). Heterozygous state for MTHFR C677T mutation was found in 5 (17.9%) patients (controls: 0.2% homozygous, 13.6% heterozygous, p>0.05). Plasma homocysteine did not correlate with extent, severity or duration of disease, or with MTHFR C677T heterozygous state, but correlated with serum folic acid level (p=0.003) and BMI (p=0.03). With folate supplementation, homocysteine decreased significantly in patients who had hyperhomocysteinemia at baseline. Hyperfibrinogenemia was detected in 3 patients (none in 100 controls). Plasma fibrinogen was not affected by duration, extent or severity of UC and did not decrease with remission of disease. Only one patient had heterozygous factor V Leiden mutation.

Conclusion: Vascular thrombosis occurred in less than a fifth of the UC population studied. Hyperhomocysteinemia reversible by folate supplementation and hyperfibrinogenemia were observed, but their contribution and that of factor V Leiden mutation appear to be insignificant.

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products within 2 weeks (2 patients) were excluded. Other exclusion criteria for the protocol were kidney disease, pregnant and lactating women, patients who had undergone recent surgical intervention (<3 months), malignancy, and patients on parenteral nutrition, but no patient met these criteria.

The hospital ethics committee approved the study and all subjects provided written informed consent.

Investigations
Colonoscopy was done with fiberoptic colonoscope (FC100 MR; Fujinon, Japan) at baseline and multiple biopsies were obtained for histology. Severity of disease was assessed clinically (Truelove and Witt’s criteria). Extent of disease was assessed using colonoscopy and histology. In the event of difference, histological extent was taken for analysis.

Patients received folic acid 5 mg daily after the baseline samples were collected. All patients received mesalamine (Mesacol; Sun Pharmaceuticals, India) in a dose of 2400 mg daily in active disease and 1200–2400 mg daily in remission. Glucocorticoids (prednisolone or IV hydrocortisone), azathioprin and cyclosporin were given when clinically indicated. One patient underwent colectomy for severe disease during the study.

Patients were followed up as outpatients for a median period of 8 months. Remission was defined as no or mild symptoms and grade 1 or 2 severity on repeat sigmoidoscopic examination. Investigations at 2 months into remission were possible in only 21 patients as one patient died in the postoperative period, three patients did not follow up, and samples in 3 patients could not be analyzed due to technical reasons.

Complete hemogram, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum total protein and albumin, renal and liver function tests, and serum folic acid and B12 levels (chemiluminescence immunoassay; ADVIA Centaur; Bayer, USA) were obtained at baseline and at two months into remission.

Nine milliliters of blood was also collected in 3.13% trisodium citrate and 2 mL in EDTA after overnight fast at baseline before any medication was started and at 2 months into remission. The plasma and serum samples were stored at ~80°C until analyzed. Serum homocysteine was estimated using a commercial ELISA kit (Bio-Rad Labs, USA). Plasma fibrinogen was estimated using the FIBRI PREST kit (Diagnostica Stago, Asnieres-Sur-Seine, France).

DNA was extracted from the peripheral blood leukocytes as described. Factor V Leiden (G1691A) and MTHFR (C677T) mutations were studied by polymerase chain reaction by using specific primers followed by digestion with restriction enzymes MnlI and Hinf, respectively. The digestion fragments were resolved in 10% polyacrylamide gel followed by ethidium bromide staining and were visualized under ultraviolet light.

Controls
Data available with the laboratory from healthy historical controls for homocysteine (n=100), MTHFR C677T (n=500) and fibrinogen (n=100) were used for comparison.

Statistics
Data are presented as mean, range and proportions. Chi square test or Fisher’s exact test was used to compare proportions. Two-tailed Student’s t test for paired data was used to compare baseline and follow-up measurements in patients. Comparison with healthy controls was performed with Student’s t test for unpaired data, or Mann Whitney U test and ANOVA as appropriate. Correlation was tested using Pearson’s correlation (r). In all statistical procedures, p<0.05 was considered significant.

Results
Baseline characteristics of screened and studied patients are summarized in the Table.

Over a median follow up of 8 months, 3 of 86 (3.5%) patients developed deep venous thrombosis; these patients could not be tested for procoagulant status. One patient (1.2%) had mesenteric and portal venous thrombosis at presentation; this patient was negative for factor V Leiden mutation, but the other tests could not be done as the patient had received heparin and multivitamins. All these
patients had active disease at the time of venous thrombosis. Stenosis at the origin of celiac axis was observed in one patient on angiography done to rule out mesenteric ischemia.

Folic acid deficiency was seen in 6 (21.4%) of the 28 patients studied, while 2 patients were deficient in vitamin B12; one patient had deficiency of both. Five patients had hemoglobin <10 g/dL: one of them had megaloblastic anemia with MCV 105 fl, while the others had microcytic hypochromic anemia.

Homocysteine

Hyperhomocysteinemia (serum homocysteine >15 μmol/L) was detected in 11 (39.3%) of 28 patients. This was significantly more frequent than in the control population (15/100, 15%, p<0.007). Homozygous state for MTHFR C677T variant was not detected in any patient, while heterozygous state was found in 5 patients (17.9%). This finding was similar in the control population (1 [0.2%] homozygous and 68 [13.6%] heterozygous C677T variant). Homocysteine levels correlated significantly and negatively with serum folate level (r = -0.54, p=0.003) and positively with BMI (r = 0.4, p<0.05). BMI (r = 0.2, p=0.2) or serum B12 levels (r = 0.25, p=0.19). The mean (SD) homocysteine level (14.43 [7.6] μmol/L) as well as frequency figures are likely to be underestimates as only symptomatic patients were studied, while 2 patients were deficient in vitamin B12; one patient had deficiency of both.

Repeat tests were done at 8 weeks into remission. In patients who had hyperhomocysteinemia at baseline (n=10), the homocysteine level decreased from 21.7 (SD 6.0) μmol/L to 14.7 (6.2) μmol/L (p=0.03; data of one patient were not available). Two patients who had normal homocysteine levels at baseline developed hyperhomocysteinemia at follow up.

Fibrinogen

Hyperfibrinogenemia (plasma fibrinogen >400 mg/dL) was seen in 3 patients as against none in the control group. The mean plasma fibrinogen was similar in patients (303.3 [100.7] mg/dL; n=28) and in controls (270.5 [41.3] mg/dL; n=100; p>0.05). The mean plasma fibrinogen was similar during activity (281.8 [70.0] mg/dL, n =17) and in remission (258.2 [66.5] mg/dL; p=0.26).

Although ESR and CRP showed significant correlation with each other, fibrinogen level did not correlate with these acute-phase reactants (r = -0.098, p=0.6, and r = 0.25, p=0.2), or with age (r = -0.3, p=0.1), gender (mean [SD] fibrinogen level in male and female patients 294.8 [84.6] mg/dL and 321 [132.4] mg/dL, respectively; p=0.8), BMI (r = - 0.19, p=0.3), disease extent (proctosigmoiditis 264.1 [52] mg/dL, left-sided colitis 351.9 [130] mg/dL, pancolitis 277 [69] mg/dL, p=0.2) and severity (mild 303 [118.7] mg/dL, moderate 294.7 [68.9] mg/dL, severe 320 [146] mg/dL, p=0.9).

Factor V Leiden mutation

Only one (3.4%) patient was heterozygous for this mutation. The patient who had portal and mesenteric thrombosis was negative for this mutation.

Discussion

Patients with UC may experience systemic thromboembolic events. In a large series from the Mayo Clinic, systemic thromboembolism occurred in 92 (1.3%) of 7199 patients with inflammatory bowel disease. Two Indian studies reported vascular thrombosis (deep venous thrombosis, Budd Chiari syndrome) in 2% of patients with UC. The occurrence of deep venous thrombosis in 3 (3.5%) patients and portal and mesenteric venous thrombosis in one patient in our study was higher than in the two previous Indian series. Our study was a longitudinal one with median duration of follow up of 8 months, while the previous two studies were cross-sectional. However, these figures are likely to be underestimates as only symptomatic vascular involvement was investigated.

It is well established that inflammation itself can activate the coagulation cascade. Many plasma coagulation factors are elevated in inflammation (acute-phase response). In our study, hyperfibrinogenemia was present in 3 of 28 patients (10.7%) with UC. Although many previous studies have noted higher fibrinogen level in active disease, we found no correlation of disease duration, severity and extent and levels of acute-phase reactants (ESR, CRP) with fibrinogen level. Moreover, there was no significant difference between the levels of fibrinogen in patients with moderate to severe ulcerative colitis and during remission. This suggests that the hyperfibrinogenemia that occurs in UC may not be an acute-phase reaction, but may be a primary phenomenon.

In many studies, elevated fibrinogen levels correlated with disease activity and the level of acute-phase reactants, suggesting that moderately elevated fibrinogen levels may simply report a state of inflammation. On the other hand, hyperfibrinogenemia, as observed in our study, could alter the hemodynamic properties of blood or enhance concentration-driven enzyme-substrate interaction between thrombin, fibrinogen and platelets, and thus lead...
Hyperhomocysteinemia and hyperfibrinogenemia in IUC

Hyperhomocysteinemia is reported to be an independent risk factor for arterial as well as venous thrombosis. It can be caused either by genetic defects in the enzymes involved in homocysteine metabolism (remethylation or trans-sulfuration pathways) or by nutritional deficiencies in vitamin cofactors, such as vitamin B12, vitamin B6, and folic acid. Methylene tetrahydrofolate reductase is a critical enzyme involved in the remethylation pathway of homocysteine metabolism. A common mutation (C to T substitution at nucleotide 677, alanine to valine) has been identified in the MTHFR gene. Homozygosity for these polymorphisms is a cause of moderate hyperhomocysteinemia, but its direct role in determining venous thrombosis is controversial.

The reported frequency of hyperhomocysteinemia in inflammatory bowel disease varies between 11% and 52%, which is significantly higher than in the control population (3.3% to 5%). We found hyperhomocysteinemia in 11 patients (39.3%). This was significantly higher than in our control population (15%). The frequency of hyperhomocysteinemia and mean homocysteine level was not affected by MTHFR C677T mutation. Also, MTHFR gene mutation was seen with similar frequency in patients with UC and the control subjects. Homocysteine level did not correlate with the duration, severity or extent of UC in our study.

Folate supplementation resulted in correction of the hyperhomocysteinemia. The prevalence of hyperhomocysteinemia in our control population was higher than that reported in Western studies. This may be because of higher prevalence of nutritional deficiencies in the Indian population. Indeed, in patients with inflammatory bowel disease, inadequate nutritional intake, excessive nutrient requirement, and possible folate and B12 malabsorption in those who had undergone intestinal resection or had involvement of the terminal ileum are likely to accentuate the folate and vitamin B12 deficiency. The practice of restricting milk in vegans may also accentuate vitamin B12 deficiency in patients with UC.

We assessed folate status by measuring serum folic acid level. However, folate level is highly labile, with normalization even with a single folate-rich nutritious meal while hospitalized. Though we tried to limit this factor by obtaining serum folate level as outpatients or by obtaining the samples as early as possible when admitted, our observation of folate acid deficiency in 7 (25%) patients might be an underestimate.

Various studies have shown an inverse correlation between homocysteine and folate or vitamin B12 plasma concentrations and the efficacy of vitamin supplementation in the lowering of plasma homocysteine levels. In our study, we could not demonstrate any correlation between vitamin B12 and homocysteine. This might be due to the small number of patients (only three) who had vitamin B12 deficiency, although Papa et al also had similar observation in patients with ulcerative colitis. Only one patient had megaloblastic anemia, suggesting that folate deficiency largely may be subclinical in patients with UC.

Another interesting observation was the significant correlation of body mass index with serum homocysteine. This was not observed in earlier studies. Nevertheless, it could help explain higher prevalence of vascular disease in obese patients.

In conclusion, vascular thrombosis occurred in less than 5% of the UC population we studied. Hyperhomocysteinemia reversible by folate supplementation and hyperfibrinogenemia were observed, but their contribution and that of factor V Leiden mutation appear to be insignificant.

References
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