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BRIEF ARTICLE

Increased levels of homocysteine in patients with ulcerative colitis

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Abstract

AIM: To investigate serum levels of homocysteine (Hcys) and the risk that altered levels carry for thrombosis development in ulcerative colitis (UC) patients.

METHODS: 55 UC patients and 45 healthy adults were included. Hcys, vitamin B12 and folic acid levels were measured in both groups. Clinical history and thromboembolic events were investigated.

RESULTS: The average Hcys level in the UC patients was 13.3 ± 1.93 μmmol/L (range 4.60-87) and was higher than the average Hcys level of the control group which was 11.2 ± 3.58 μmmol/L (range 4.00-20.8) ($P < 0.001$). Vitamin B12 and folic acid average values were also lower in the UC group ($P < 0.001$). When multivariate regression analysis was performed, it was seen that folic acid deficiency was the only risk factor for hyperhomocysteinemia. Frequencies of thromboembolic complications were not statistically significantly different in UC and control groups. When those with and without a thrombosis history in the UC group were compared according to Hcys levels, it was seen that there were no statistically significant differences. A negative linear relationship was found between folic acid levels and Hcys.

CONCLUSION: We could not find any correlations between Hcys levels and history of prior thromboembolic events.

Key words: Ulcerative colitis; Homocysteine; Folate; Vitamin B12

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INTRODUCTION

Inflammatory bowel diseases (IBD) are chronic disorders of unknown etiology characterized by destructive inflammation of the gastrointestinal tract, acute exacerbations and remission periods. IBD is exemplified by two major groups of diseases. These include ulcerative colitis (UC) and Crohn's disease (CD). These two diseases are indistinguishable in 10%-15% of IBD patients. This is termed

Indeterminate Colitis (IC)[1]. Patients with IBD are at increased risk of experiencing thromboembolic complications. The incidence of arterial and venous thromboembolic diseases has been reported to be 1%-8% in UC and CD, however some autopsy studies have reported this incidence to be as high as 39%[2,3]. In the study of Bernestein *et al*[4], the risks of deep vein thrombosis and pulmonary emboli were found to be 3 times higher than that of the general population. In addition, venous thrombosis occurs earlier in IBD patients compared to the general population[5]. Thromboembolism is a multifactorial condition. Studies have indicated a state of hypercoagulability involving all the components of the coagulation system in patients with UC[6-8]. The exact etiology and pathogenesis of hypercoagulability have not been explained, however it is widely accepted that this condition is associated with acquired or genetic defects of the coagulation system and the procoagulant effect of proinflammatory cytokines[9-14]. This hypercoagulability increases the risk of thromboembolic events and has a role in the pathogenesis of UC *via* formation of microthrombi in the intestinal microcirculation[15,16].

Homocysteine (Hcys), which was first defined in 1932 by Butz and Vigneaud, is a sulphur-containing amino acid synthesized during transformation of methionine to cysteine in methionine metabolism[17]. Mild hyperhomocysteinemia occurs in about 5%-7% of the general population[18-24]. Increased Hcys levels are due to enzyme abnormalities in metabolic pathways or to nutritional deficits, including folate and vitamin B2, B6 and B12 deficiencies[25,26]. The exact relationship between hyperhomocysteinemia and induction of thrombosis is unknown, however, a state of hypercoagulability resulting from endothelial dysfunction has been blamed[26,27].

Vitamin B12 and folic acid deficiencies due to malnutrition, malabsorption and antifolate medications including methotrexate and sulphasalazine are quite common in IBD. The deficiency of these key nutrients leads to increased Hcys levels in IBD. Several recent studies have reported increased Hcys levels in IBD[28-33]. The objective of this study was to determine levels of homocysteine, folic acid and vitamin B12 in patients with UC; to compare these data with those of a healthy control group; and to investigate the relationship between homocysteine levels and disease activation, thromboembolic complications, localization of disease, and levels of folic acid and vitamin B12.

MATERIALS AND METHODS

Subjects

A total of 55 UC patients [38 males, 17 females; mean age (± SD) 47.4 ± 13.80, age range 20-78 years] were recruited in the study. The patient group consisted of patients followed up and treated for IBD at the outpatient clinic between the dates March 2006 and January 2007. Diagnosis of UC was established using clinical, endoscopic and histological criteria. All patients underwent appropriate endoscopy (colonoscopy or rectosigmoidoscopy) to determine endoscopic activity indices and localization of disease. Biopsy samples were obtained for histopathologic examination when necessary. Rachmilewitz Endoscopic Activity Index was used to determine the degree of endoscopic activity[34]. Clinical activity index was determined according to Truelove and Witts criteria[35].

Current complaints, duration of disease, extraintestinal symptoms, smoking status, history of thromboembolic events (deep vein thrombosis, pulmonary emboli, myocardial infarction, stroke and peripheral arterial obstruction) and medications were recorded. Patients with any other systemic disorders including diabetes mellitus, hyperthyroidism, chronic liver and renal disease, and history of cancer, as well as patients on vitamin B12, folic acid and oral multivitamin supplements, were excluded from the study.

A detailed clinical history was obtained from all patients regarding any previous events of arterial or venous thrombosis. A total of 3 subjects (5.5%) had history of arterial and venous thromboembolic events. Two of these (3.63%) were peripheral deep vein thrombosis, and 1 (1.81%) was myocardial infarction (deep vein thrombosis was diagnosed using Doppler USG, and myocardial infarction using ECG and cardiac enzymes).

A healthy control group was composed of patients with similar age and sex distribution, and without any metabolic, neoplastic or inflammatory diseases or any history of thromboembolic diseases. The healthy control group consisted of a total of 45 healthy individuals [31 males, 14 females; mean age (± SD) 46.4 ± 13.89, age range 20-77 years].

Blood samples

Blood samples were obtained from all patients following 12-h fasting and these samples were used for complete blood count, sedimentation rate, C reactive protein (CRP), routine biochemical analysis (including total cholesterol, triglycerides, LDL cholesterol, VLDL cholesterol), thyroid function tests, folic acid, vitamin B12, homocysteine level examinations. Biochemical parameters were measured using standard methods. Serum levels of folic acid and vitamin B12 were measured using chemiluminescent microparticle immunoassay (CMIA) (Abbott System). Serum homocysteine levels were measured with high pressure liquid chromatography (HPLC) (Betamed, Agilent 1100 series, Chromosystems Reagent Kit).

Statistical analysis

Data were analyzed using SPSS 11.5 package software. Definitive statistics were indicated as mean ± SD or median (minimum-maximum) in continuous variables, whereas categorical data were expressed as number of observations (%). Presence of any significant differences between case and control groups in terms of measurements was evaluated using Mann Whitney *U* test. In contrast, Kruskal Wallis was used to determine any significant differences between treatment types, clinical activity index and localization groups in terms of Hcys levels. Spearman correlation test was used to determine the asso-

Table 1 Demographic features of UC and control groups

	Patients with UC (n = 55)	Healthy controls (n = 45)	P
Mean age (yr ± SD)	47.4 ± 13.80	46.4 ± 13.89	0.484
Gender (male/female)	38/17	31/14	0.983
Smoking status (%)	17 (30.9)	15 (33.3)	0.796
Localization (%)			
Rectum/sigmoid	34 (61.8)		
Left colitis	12 (21.8)		
Pancolitis	9 (26.4)		
Activity of UC (%)			
Active	28 (50.9)		
Inactive	27 (49.1)		
Medical treatment (%)			
5-ASA	41 (74.5)		
Steroids	3 (5.5)		
AZA	11 (20.0)		

UC: Ulcerative colitis; ASA: Aminosalicylic-acid; AZA: Azathioprine.

ciation of Hcys levels and duration of disease, clinical activity index, vitamin B12, folic acid and CRP. χ^2 or Fisher's exact test was used for categorical comparisons. A P level of < 0.05 was accepted for statistical significance.

RESULTS

Fifty-five patients with UC were recruited into the study. Patients consisted of 38 (69.1%) males, 17 (30.9%) females and mean age was 47.4 ± 13.80 years with a range between 20-78 years. Forty-five healthy individuals were recruited in the control group, consisting of 31 (68.9%) males, 14 (31.1%) females, with a mean age of 46.4 ± 13.89 years, range between 20-77 years. Groups did not differ statistically significantly in terms of age, sex and smoking status (P = 0.484, P = 0.983, P = 0.796, respectively).

Analysis of distribution of disease localizations in the UC group indicated that 34 (61.8%) patients had distal involvement, 12 (21.8%) patients had left colitis and 9 (16.4%) had pancolitis. Clinical activity indices of patients in the UC group showed that mild, moderate and severe cases were 28 (50.9%), 21 (38.2%) and 6 (10.9%) in number. Analysis of current treatment indicated that 41 patients (74.5%) received 5-ASA, 11 (20%) received azothioprine and 3 (5.5%) received corticosteroid treatment (Table 1).

Mean homocysteine level was 13.3 ± 1.93 μmol/L (range 4.60-87) in the UC group and 11.2 ± 3.58 μmol/L (range 4.00-20.8) in the control group ($P < 0.001$). Mean serum folic acid level was 5.1 ± 2.19 ng/mL (range 1.90-10.90) in the UC group and 6.3 ± 0.87 ng/mL (range 5-10) in the control group ($P < 0.001$). Mean level of vitamin B12 was 250.4 ± 82.49 pg/mL (range 96-505) in the UC group and 327.4 ± 73.90 pg/mL (range 200-482) in the control group ($P < 0.001$).

Thromboembolic events had similar prevalence in UC and control groups and did not differ statistically significantly in this respect (P = 0.250) (Table 2). It was determined that a total of 3 (5.5%) subjects had a history of arterial and venous thromboembolic events. Two

Table 2 Hcys, folic acid and vitamin B12 levels in UC and control groups (95% CI)

	Patients (n = 55)	Controls (n = 45)	P
Hcy (μmol/L)	13.3 ± 1.93	11.3 ± 3.58	< 0.001
Folic acid (ng/mL)	5.1 ± 2.19	6.3 ± 0.87	< 0.001
Vitamin B12 (pg/mL)	250.4 ± 82.49	327.4 ± 73.90	< 0.001
Thromboemboli	3 (5.5%)	0 (0%)	0.250

Table 3 Correlation between Hcys and other variables among patients with UC

	Relationship coefficient	P
Disease duration	0.105	0.447
Folic acid	-0.311	0.021
Vitamin B12	-0.146	0.288
CRP	0.244	0.073
Clinical activity index	0.115	0.403

of these (3.63%) were peripheral deep vein thrombosis and 1 (1.81%) was myocardial infarction (diagnoses were established using Doppler USG for deep vein thrombosis and ECG plus cardiac enzymes for myocardial infarction). The UC group did not differ from control to a statistically significant degree (P = 0.250). These three patients had normal Hcys levels.

No directly proportional relationship was determined in the UC group between homocysteine levels and disease duration, vitamin B12, CRP and clinical activity index; however, there was a negatively proportional relationship found between folic acid and homocysteine levels (r = -0.311 and P = 0.021) (Table 3).

There were no statistically significant differences within the disease localization groups of UC in terms of homocysteine levels (P = 0.096). Additionally, there were no statistically significant differences within the clinical activity index groups of UC in terms of homocysteine levels (P = 0.698). Similarly, there were no statistically significant differences within the treatment groups of UC in terms of homocysteine levels (P = 0.695).

DISCUSSION

Studies have shown that the risks of deep vein thrombosis and pulmonary emboli are three times higher in patients with IBD compared to the general population[4]. The exact pathogenesis of thromboembolism is not known and considered to be multifactorial[36-38]. Moderate hyperhomocysteinemia (hHcy) has been shown to be one of the independent risk factors associated with development of arterial and venous thrombosis[24,39-44].

Elevated Hcys might be due to genetic factors (MTHFR mutation), nutritional factors (deficiencies of folic acid, vitamin B6, vitamin B12) or medications (salazopyrin, methotrexate, corticosteroids)[28,45]. The most common cause of hHcy has been found to be folic acid deficiency in patients with UC[31,46,47].

Several previous studies also reported higher Hcys levels in patients with UC[28-33,45,48,49]. In our study, serum Hcys levels were higher in patients with UC than in healthy controls. Increased Hcys levels were negatively correlated with lower vitamin B12 and folic acid levels. However, multivariate regression analysis demonstrated that decreased folic acid levels were the most significant parameter in determining increased Hcys levels. In a similar study by Mahmut *et al*[28], increased Hcys levels were found to be associated particularly with folic acid deficiency. Hcys levels were observed to decrease following folate replacement and the authors recommended that folic acid levels should be determined and prophylactic folate treatment should be initiated in all patients with IBD. Zezoz *et al*[50] performed a similar study and found that the prevalence of increased Hcys was higher in the UC group compared to the control group (prevalence of Hcys was 30% in UC patients and 10% in control group). Statistical analysis showed that male sex, decreased folic acid and vitamin B12 levels were indicators of hHcy in patients with UC and that decreased folic acid levels were the most important factor. In our study also, decreased folic acid level was found to be the most important indicator of hHcy.

Folic acid deficiency might be associated with several factors in patients with IBD. These include inadequate intake, increased consumption or folate malabsorption in patients using medications, including particularly sulphasalazine (SASP)[30]. In the study of Zezos *et al*[50], one patient who was receiving SASP was found to have increased Hcys levels. An antifolate effect of SASP has also been demonstrated in other studies[28,29]. Only one patient was receiving SASP in our study and this patient had low folic acid and high Hcys levels.

Vitamin B12 levels were found to be decreased in our UC group compared to our control group; however this finding did not attain statistical significance. Studies have shown normal vitamin B12 levels among patients with increased Hcys levels[32,47]. The study of Romagnuolo *et al*[32] performed with regard to this issue has indicated that the prevalence of hHcy was 15.4% in IBD patients and that 80% of these patients had normal vitamin B12 levels. These authors suggested that there could be significant deficiency of vitamin depots or subclinical vitamin B12 deficiency in IBD patients with increased homocysteine and normal serum vitamin levels. In the study by Lambert *et al*[47], vitamin B12, B6 and folate deficiencies were determined as the most sensitive indicators of hHcy. These authors also suggested that subclinical vitamin deficiencies or intracellular vitamin deficiencies heralded vitamin deficiencies in circulation and that multivitamin (including folate) supplements were effective in decreasing Hcys levels. Similar to several previous studies, we found that hHcy did not correlate with disease activation, localization and medications[28,29,31,33]. However, some studies have shown a statistically significant relationship between disease activation and hHcys and reported that Hcys levels were markedly higher in patients with active disease compared to those with inactive disease[49,51].

Two recent studies have investigated Hcys levels in colon mucosa and aimed to discover the role of Hcys in the pathogenesis of UC[52,53]. Morgenstern *et al*[52] obtained biopsy samples from transverse colon and sigmoid or descending colon mucosa of 11 UC and 5 CD patients and examined these samples using high performance liquid chromatography (HPLC). Concentrations of Hcys were found to be higher in the UC and CD patients compared to healthy individuals. This study is the first to demonstrate elevated Hcys levels in human colon mucosa. No statistically significant differences were determined between Hcys levels, and disease activation or current treatment (particularly those receiving and not receiving sulphasalazine) in IBD patients. Interestingly, patients with other chronic inflammatory bowel diseases had normal Hcys levels; and patients with history of colorectal cancer had higher Hcys levels in colon mucosa compared to the normal[52]. The incidence of colon cancer among patients with UC is about 8 times higher than sporadic colon cancer. Hcys has mitogenic and proliferative features[54]. Elevated Hcys levels have been reported to be a risk factor for carcinogenesis[52].

In their study, Danese *et al*[53] measured both plasma and colon mucosal Hcys levels concomitantly in patients with IBD. They reported that Hcys levels were increased in both plasma and colonic mucosa of patients with UC and CD and suggested that Hcys could have a proinflammatory role in IBD. Similar to our study, this investigation did not determine any correlation between Hcys levels and disease activation or medications. A negative correlation was determined between circulatory and mucosal Hcys levels and folic acid levels, emphasizing the necessity of folic acid supplementation in patients with IBD[53].

Large retrospective studies have shown the rate of thromboembolic complications in patients with IBD to be between 1.3%-6.4%[55,56]. Oldenburg *et al*[29] reported that the rate of complications in a total of 231 patients with IBD was 5.7% in those with CD, and 9.0% in those with UC. Comparison of patients with and without venous thrombosis did not yield any statistically significant difference with regard to Hcys concentration. They suggested that hHcy did not influence the prevalence of thrombosis in patients with IBD; but that thrombosis developed secondary to multifactorial phenomena. These authors also suggested that hHcy could contribute to the pathogenesis of thromboembolic events in some of these patients[29].

Papa *et al*[33] reported the rate of thromboembolic complications to be 9.4% (6 patients) in 39 UC and 25 CD patients. There was hHcy in 2 of these patients. There was no statistically significant difference compared to the control group. Additionally, no statistically significant differences were found in Hcys levels in the within group comparison of IBD patients with regard to history of previous thromboembolic events[33]. In our study there were 3 patients with history of thromboembolic events and no statistically significant differences were present compared to the control group. We concluded that hHcy is not the major factor contributing to the development of thromboembolic complications; and that multifactorial etiology was more relevant.

In conclusion, hyperhomocysteinemia is a common

phenomenon in patients with IBD. Vitamin deficiencies should be determined in all patients with IBD and folate and vitamin B complex supplementations should be included in their treatment. In our study, we could not find any correlation between Hcys levels and history of arterial and venous thrombosis. Further studies should be performed to investigate the multifactorial etiology in the development of thromboembolic events in patients with IBD.

COMMENTS

Background

Patients with inflammatory bowel diseases (IBD) are at increased risk of experiencing thromboembolic complications. Studies have indicated a state of hypercoagulability involving all the components of the coagulation system in patients with ulcerative colitis (UC). The exact etiology and pathogenesis of hypercoagulability have not been explained, however, it is widely accepted that this condition is associated with acquired or genetic defects of the coagulation system and with the procoagulant effect of proinflammatory cytokines.

Research frontiers

In their study, Danese *et al* measured both plasma and colon mucosal homocysteine (Hcys) levels concomitantly in patients with IBD. They reported that Hcys levels were increased in both plasma and colonic mucosa of patients with UC and Crohn's disease (CD) and suggested that Hcys could have a proinflammatory role in IBD.

Innovations and breakthroughs

The results of studies investigating the influence of hyperhomocysteinemia on thrombosis in patients with IBD are controversial. Some studies suggest that it could contribute to the pathogenesis of thrombosis whereas other authors suggest that thrombosis develops in IBD patients secondary to multifactorial phenomena.

Applications

In this study, the authors found that the average Hcys level in UC patients was significantly higher than the average Hcys level of the control group. They could not find any correlation between Hcys levels and history of arterial and venous thrombosis. No directly proportional relationship was determined in the UC group between homocysteine levels and disease duration, vitamin B12, CRP level and clinical activity index; however there was a negatively proportional relationship found between folic acid and homocysteine levels. Vitamin B12 and folic acid average values were also lower in the UC group ($P < 0.001$). When multivariate regression analysis was performed, it was seen that folic acid deficiency was the only risk factor for hyperhomocysteinemia. For this reason the authors think that vitamin deficiencies should be determined in all patients with IBD and folate and vitamin B complex supplementation should be included in their treatment.

Terminology

Homocysteinemia is defined as elevation of homocysteine (a sulphur-containing amino acid) level in blood. It is an established risk factor for cardiovascular diseases and premature atherosclerosis.

Peer review

The study demonstrates increased levels of Hcys related to a decrease of folic acid and B12 in patients affected by UC. These data are not associated with an increase of thromboembolic events. The manuscript is well structured.

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