Journal of Crohn's and Colitis (2011) 5, 110–114

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Prevalence and risk factors of hyperhomocysteinemia in Tunisian patients with Crohn's disease

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Received 11 October 2010; received in revised form 28 October 2010; accepted 29 October 2010

KEYWORDS
Crohn's disease;
Folic acid;
Homocysteine;
Vitamin B_{12}

Abstract

Background and aims: The role of hyperhomocysteinemia (HHC) and its determinants in Crohn's disease (CD) remain uncertain. This study was aimed to determine the prevalence of HHC and its main risk factors in Tunisian patients with CD.
Methods: This study included 89 patients with CD and 103 age- and sex-matched healthy subjects. Fasting venous blood was collected in all subjects allowing the assessment of homocysteine, folate, vitamin B_{12}, C-reactive protein and creatinine levels. Logistic regression models were applied to identify factors associated with HHC in CD patients.
Results: Plasma homocysteine was higher (13.69 ± 4.84 μmol/l vs. 10.77 ± 2.80 μmol/l; $p<0.01$) and HHC was more frequent (31.5% *vs.* 7.8%; $p<0.001$) in patients compared with controls. The association between HHC and CD persisted after adjustment for smoking, body mass index and serum folate, vitamin B_{12}, creatinine and C-reactive protein. In patients with CD, multivariate analysis showed that HHC was positively associated with age [multi-adjusted odds-ratio (95% confidence interval): 1.14 (1.06–1.24); $p<0.001$], active disease [7.54 (1.15–49.3); $p=0.03$], disease duration >2 years [8.69 (1.53–49.3); $p=0.02$] and inversely related to plasma folate [0.64 (0.48–0.84); $p=0.002$] and vitamin B_{12} (0.993 (0.987–0.999); $p=0.02$].
Conclusion: HHC is common in Tunisian patients with CD and is related to B vitamins deficit, as well as disease activity and duration. Further studies should test the effect of correction of HHC by vitamin B supplementation on progression and complications of CD.

1. Introduction

Crohn's disease (CD) is a chronic inflammatory bowel disease (IBD) of unknown origin. Its evolution can be riddled with intestinal and extra-intestinal complications, particularly atherothrombotic events. Homocysteine (Hcy) is a sulfur

Abbreviations BMI, body mass index; CD, Crohn's disease; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; Hcy, homocysteine; HHC, hyperhomocysteinemia; IBD, inflammatory bowel disease

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doi:10.1016/j.crohns.2010.10.010

amino acid that has toxic properties on the vascular wall. Increased plasma Hcy concentration or hyperhomocysteinemia (HHC) has been established as a risk factor for arterial and venous thrombosis in general population.[1,2] Numerous studies have shown that HHC is common in patients with IBD[3–18] and some authors have suggested an involvement of Hcy in the pathogenesis of these diseases.[16] It has been demonstrated that HHC observed in IBD was often associated with folate, vitamin B_{12} or vitamin B_6 deficiencies. However, there are discrepancies on the role of other factors, such as disease characteristics, administrated drugs and intestinal resection, as well as the contribution of HHC in increased risk of thromboembolism in CD. This study was aimed to determine the prevalence of HHC and to clarify its main determinants in a group of Tunisian patients with CD.

2. Subjects and methods

2.1. Subjects

This case–control study included consecutive outpatients and hospitalized patients with CD followed in the Department of Gastroenterology (Rabta hospital of Tunis) from May to December 2007. Pregnant women, patients with extensive small bowel resection (>60 cm of small bowel) or those having received folate or vitamin B12 supplementation during the six previous months were excluded from the study. In total, 89 patients, 47 men (52.8%) and 42 women (47.2%), aged 18 to 65 years were included. A control group of 103 apparently healthy subjects matched for age and sex were recruited among hospital staff and their relatives in the same period. For each patient, the following data were recorded: age, gender, weight, height, smoking status, CD duration, topography of intestinal lesions, severity of the attacks measured by Crohn's Disease Activity Index at baseline (CDAI), with CDAI<150, inactive; 150–219, mild; 220–450 moderate and ≥450, severe disease, medical treatment and history of bowel resection. Fasting venous blood samples were collected for all subjects. The study protocol was approved by the ethics committee of the Rabta hospital of Tunis and all participants gave their informed consent to participate.

2.2. Analytical methods

Plasma Hcy, vitamin B_{12} and folate were measured by specific immunochemical methods by an Axsym auto analyzer using Abbott reagents (Abbott Laboratories, Illinois, USA). Creatinine and C-reactive protein (CRP) were measured by colorimetric method and immunoassay, respectively, on a Hitachi 912 auto analyzer using Roche reagents (Roche Diagnostics GmbH, Mannheim, Germany). Body mass index (BMI) was calculated as follows, BMI (kg/m^2) = weigh/heigh^2. HHC was considered for plasma Hcy > 15 μmol/l, matching with the 90th percentile value in the control group.

2.3. Statistical analysis

Statistical analysis was performed using SPSS for Windows, version 15.0 (SPSS Inc., Chicago, USA). Comparison of means was performed by Student *t* test or Mann–Whitney test in case of unequal variances or non-Gaussian distribution. Association between categorical variables was tested by chi-squared test. The relationship between continuous variables was tested by Spearman correlation coefficient. To verify the independence of association between HHC and CD, we applied a binary logistic regression model with HHC as response variable, with CD and major determinants of plasma Hcy as independent variables. To identify factors associated with HHC in patients with CD, a backward logistic regression model was applied with HHC as response variable and several parameters related to patient or disease as confounding factors. Goodness-of-fit of multivariate models was satisfactory. The statistical level of significance was established at 5%.

3. Results

The main characteristics of disease in our CD patients are given in Table 1. Plasma Hcy was higher and HHC was more frequent in patients compared to controls (Table 2, Fig. 1). Vitamin B_{12} levels were lower among patients, but folate levels were comparable between patients and controls. Plasma CRP and the prevalence of smoking were higher, but BMI and serum creatinine were lower in patients (Table 2). The association between HHC and CD remained significant ($p=0.003$) after adjustment for smoking, BMI and serum folate, vitamin B_{12}, creatinine and CRP (Table 3).

In patients with CD, there was a weak to moderate negative correlation of Hcy with vitamin B_{12} ($r=-0.43$, $p<0.001$) and folate ($r=-0.22$, $p=0.03$) (Fig. 2), and a weak positive correlation with age ($r=0.28$, $p=0.008$), serum creatinine ($r=0.25$, $p=0.02$) and CRP ($r=0.23$, $p=0.03$) and erythrocyte sedimentation rate (ESR) ($r=0.21$, $p=0.05$). Compared to patients with normal plasma Hcy, those with HHC were older (40.1 ± 13.0 years *vs.* 33.1 ± 11.8 years; $p=0.01$) and had lower levels of vitamin B_{12} (226 ± 109 ng/l *vs.* 367 ± 171 ng/l; $p<0.001$). There was no significant difference in gender,

Table 1 Main characteristics of Crohn's disease patients.

Variable	Patients ($n=89$)
Illness duration (months)	64.6 ± 69.4
Active disease (%)	28.1
Localization of lesions (%)	
Extended small intestine	5.6
Ileal	13.5
Ileocolonic	38.2
Colonic	41.6
Ano-perineal exclusive	1.1
Medical treatment at inclusion	
Corticosteroids (%)	32.6
Azathioprine (%)	46.1
Sulfasalazine (%)	19.1
5-Aminosalicylate (%)	4.49
History of intestine resection	
Ileocecal resection (%)	15.7
Partial colectomy (%)	5.6
Total colectomy(%)	5.6

Data were expressed as means ± SD or percent.

Table 2 Plasma homocysteine, hyperhomocysteinemia and their main determinants in patients with Crohn's disease and controls.

	Patients ($n=89$)	Controls ($n=103$)	p
Age (years)	35.3±12.6	36.5±9.26	NS
Male gender (%)	52.8	48.5	NS
Smoking (%)	21.3	9.9	<0.001
Body mass index (kg/m^2)	22.9±4.40	25.9±3.29	<0.001
Homocysteine (μmol/l)	13.7±4.84	10.8±2.80	<0.001
Folates (μg/l)	8.54±3.04	8.10±3.11	NS
Vitamin B_{12} (ng/l)	295±160	378±170	0.001
Creatinine (mg/l)	8.46±2.08	9.64±1.44	<0.001
C-reactive protein (mg/l)	18.1±27.6	2.39±1.56	<0.001
Hyperhomocysteinemia (%)	31.5	7.8	<0.001

Data were expressed as means±SD or percent.

smoking status, ESR, serum folate, CRP and creatinine between patients with or without HHC. There was no significant difference in Hcy levels and prevalence of HHC according to duration of disease, disease activity, intensity of relapse for patients with active disease, nature of treatment or antecedent of intestine resection. However, in multivariate analysis, HHC was associated with age, active disease, duration of illness >2 years and serum folate and vitamin B_{12} levels (Table 4).

4. Discussion

This study showed plasma Hcy and prevalence of moderate HHC are significantly higher in CD patients compared to controls. In our series, 31.5% of patients exhibited HHC vs. 7.8% in controls. HHC remained significantly associated with CD after adjustment on the main factors of variation of Hcy (i.e. age, gender, smoking status, BMI and plasma folate, vitamin B12, creatinine and CRP levels). These data corroborate the hypothesis suggesting a link between HHC and CD. Indeed, several previous studies reported an association between HHC and IBD.[3–18] The prevalence of HHC in IBD ranges from 11% to 56% depending on the series,

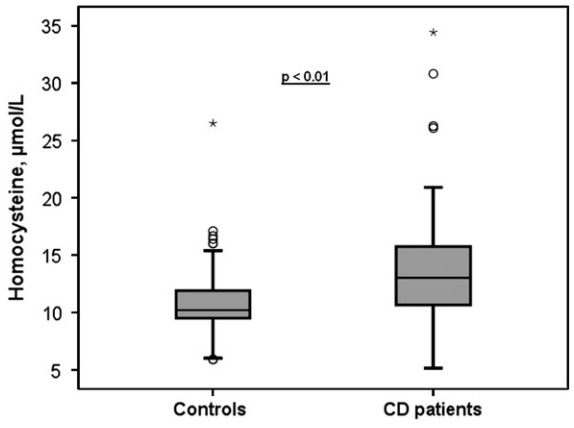

Figure 1 Comparative distribution of plasma homocysteine in patients with Crohn's disease and controls.

Table 3 Binary logistic regression model with hyperhomocysteinemia as response variable ($n=192$).

	Coefficient β	Odd ratio (95% confidence interval)	p
Crohn's disease	2.993	18.76 (2.771–126.7)	0.003
Smoking	−0.377	0.686 (0.161–2.925)	0.610
Body mass index	0.112	1.119 (0.985–1.270)	0.083
Folate	−0.358	0.699 (0.555–0.881)	0.002
Vitamin B_{12}	−0.003	0.997 (0.993–1.001)	0.120
Creatinine	0.261	1.289 (0.963–1.748)	0.087
C-reactive protein	0.009	1.009 (0.991–1.027)	0.320

with no significant difference between CD and ulcerative colitis.

HHC in patients with CD would be related firstly to nutritional origin, mainly vitamin B_{12} and/or folate deficiencies. Accordingly, numerous studies have shown an association between HHC and a low level in these vitamins in IBD.[5,6,9,10,13] In our series, multivariate analyses showed that plasma folate and vitamin B_{12} are significant determinants of HHC in patients. Deficits in these two vitamins would be multifactorial, in relation to reduced intake, intestinal malabsorption and medication, as well as increased needs for these vitamins.[19,20] The role of inflammation in HHC in IBD is controversial. HHC was found to be associated with elevated CRP in a series of 106 patients with IBD.[21] However, no association was found between Hcy and biochemical and hematological markers of inflammation in other studies.[9,22] In our study, positive correlation observed between Hcy on one hand and CRP and ESR on the other hand lost its significance in multivariate analysis. However, HHC was found to be independently associated with an active disease. In view of that, it seems that B vitamin deficits and inflammation partially contribute to HHC in CD patients. Accordingly, in the herein studies, correlations of plasma Hcy with plasma folate, vitamin B_{12}, CRP and ESR were feeble. Also, multivariate analysis showed that HHC was related to CD independently of plasma B vitamins and CRP, thereby suggesting it stems from other yet unknown factors of Crohn's. The role of genetic polymorphism of enzymes involved in Hcy metabolism was largely investigated in IBD. However, their contribution remains uncertain and may vary with populations and clinical forms.[4,12,21]

The characteristics of CD or treatments could influence Hcy levels. Drzewoski et al.[15] showed that HHC was related to disease activity, its duration, and the number of relapses. Other studies have however shown that parameters such as disease activity, extent and location of lesions, and disease duration were not associated with HHC in IBD.[14] In this study, HHC was found to be associated with disease activity and duration. Aminosalicylates have been implicated in malabsorption of folate and HHC in patients with IBD.[23] However, we did not observe any effect of treatment with sulfasalazine or aminosalicylates on plasma Hcy or folate levels. Similarly, we observed no significant effect of azathioprine on Hcy levels in our patients, consistent with the results of Maire et al.[9] The later authors reported that plasma Hcy is 2 correlated to the length of removed intestine.[9] In our study, no differences of Hcy levels or HHC prevalence were

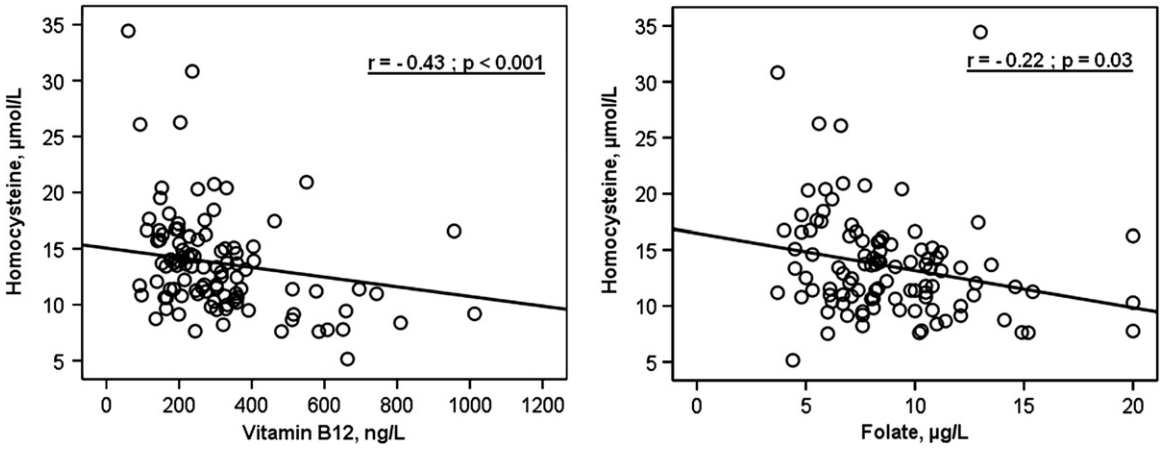

Figure 2 Correlation of plasma homocysteine and plasma vitamin B_{12} and folate in patients with Crohn's disease.

found according to antecedent of intestinal resection. Nevertheless, patients with extensive small bowel resection were excluded from the present study.

The role of HHC in increasing susceptibility to thrombosis in CD remains controversial.[4,12,24] The lack of a clear correlation reflects that hypercoagulable state in IBD would be multifactorial and suggest that HHC could be an additional factor increasing the risk of thrombosis. In our series, only three patients had an antecedent of atherothrombotic event with two having HHC. These data do not provide evidence as to the responsibility of HHC in the thrombotic tendency in CD patients.

5. Conclusion

This study showed increased prevalence of HHC in Tunisian patients with CD. The association between HHC and CD remained significant after adjustment for many factors related to the patient and the disease. Risk of HHC is all the greater as the disease is ancient and active and the B vitamin status is poor. A recent randomized trial failed to show a benefit of homocysteine lowering therapy on vascular outcomes in general population.[25] However, more studies are needed to test the effect of vitamin B supplementation on intestinal and extra intestinal complications of CD, not only atherothrombosis, but also osteoporosis and colorectal cancer.

Table 4 Backward regression model with hyperhomocysteinemia as response variable in patients with Crohn's disease (n=89).

	Coefficient β	Odds ratio (95% confidence interval)	p
Age	0.137	1.141 (1.062–1.244)	0.001
Folate	−0.451	0.642 (0.483–0.842)	0.002
Vitamin B_{12}	−0.072	0.993 (0.987–0.999)	0.020
Activity of Crohn's disease	2.023	7.546 (1.154–49.36)	0.033
Illness duration >2 years	2.161	8.692 (1.537–49.38)	0.025

Independent variables included in the model at step 1: age, gender, smoking status, body mass index, serum folate, vitamin B_{12}, creatinine and C-reactive protein, erythrocyte sedimentation rate, disease activity, illness duration >2 years, ileal localization of lesions and treatment with corticosteroids, sulfasalazine and azathioprine.

Conflict of interest statement

There are no conflicts of interest to disclose by any of the authors in relation to this study.

Acknowledgements

All authors made significant contributions in this study and were involved in drafting or revising the manuscript for content and approval of the final version submitted. The individual contribution of each co-author is as follows:

- LK and MF: study design, conducting study, data analysis/interpretation, writing the manuscript.
- WZ: conducting study, data acquisition, drafting the manuscript.
- LS: study design, conducting study, data acquisition/analysis/interpretation.
- MF and JB: study design, conducting study, reviewing the manuscript.
- NK and AF: study design, data interpretation, reviewing the manuscript.

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