World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i24.2941

World J Gastroenterol 2011 June 28; 17(24): 2941-2944
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Is hyperhomocysteinemia relevant in patients with celiac disease?

Giovanni Casella, Gabrio Bassotti, Vincenzo Villanacci, Camillo Di Bella, Fabio Pagni, Gian Luigi Corti, Giuseppe Sabatino, Mara Piatti, Vittorio Baldini

Giovanni Casella, Vittorio Baldini, Division of Internal Medicine, Desio General Hospital, 20033 Desio, Italy
Gabrio Bassotti, Giuseppe Sabatino, Gastroenterology and Hepatology Section, Department of Clinical and Experimental Medicine, Ospedale Santa Maria della Misericordia, Piazzale Menghini, 1, 06156 San Sisto (Perugia), Italy
Vincenzo Villanacci, 2nd Pathology Section, Spedali Civili, 25100 Brescia, Italy
Camillo Di Bella, Fabio Pagni, Pathology Unit, Desio General Hospital, 20033 Desio, Italy
Gian Luigi Corti, Endoscopy Unit, Division of Surgery, Desio General Hospital, 20033 Desio, Italy
Mara Piatti, Laboratory Medicine Unit, Desio General Hospital, 20033 Desio, Italy
Author contributions: Casella G, Bassotti G and Baldini V ideated and planned the study and wrote the paper; Bassotti G and Sabatino G carried out the statistical analysis; Villanacci V, Di Bella C and Pagni F analyzed the histological samples; Corti GL helped with endoscopic procedures; Piatti M helped with laboratory analyses.
Correspondence to: Gabrio Bassotti, Professor, Gastroenterology and Hepatology Section, Department of Clinical and Experimental Medicine, Ospedale Santa Maria della Misericordia, Piazzale Menghini, 1, 06156 San Sisto (Perugia), Italy. gabassot@tin.it
Telephone: +39-75-5784423 Fax: +39-75-5847570
Received: August 10, 2010 Revised: November 13, 2010
Accepted: November 20, 2010
Published online: June 28, 2011

Abstract

AIM: To investigate whether this might be related to the presence of hyperhomocysteinemia.

METHODS: From January 1998 to December 2008, we evaluated the presence of hyperhomocysteinemia in a series of 165 adult celiac disease (CD) patients (138 females and 27 males, mean age 43 years).

RESULTS: Hyperhomocysteinemia was evident in 32 patients (19.3%), although most of them had moderate levels (mean value 25 mcg/ml; range 15-30). Only one patient had a history of myocardial infarction (heterozygosis for N5-N10-metil tetrahydrofolate reductase mutation).

CONCLUSION: The systematic assessment of hyperhomocysteinemia seems, at present, unjustified in CD patients.

Key words: Celiac disease; Endoscopy; Histology; Hyperhomocysteinemia

Peer reviewers: Javier San Martín, Chief, Gastroenterology and Endoscopy, Sanatorio Cantegril, Av. Roosevelt y P 13, Punta del Este 20100, Uruguay; Andrew S Day, MB, ChB, MD, FRACP, AGAF, Associate Professor, Department of Paediatrics, University of Otago, Christchurch, PO Box 4345, Christchurch 8140, New Zealand

Casella G, Bassotti G, Villanacci V, Di Bella C, Pagni F, Corti GL, Sabatino G, Piatti M, Baldini V. Is hyperhomocysteinemia relevant in patients with celiac disease? *World J Gastroenterol* 2011; 17(24): 2941-2944 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i24/2941.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i24.2941

INTRODUCTION

Hyperhomocysteinemia, considered as an important risk factor in venous thrombosis[1-3], has a prevalence in the general population of 5%-7%[4], and causes damage of the vascular endothelium by disrupting the release of nitric oxide, an important vasodilator factor[5], followed by

platelet activation and thrombus formation[4].

Celiac disease (CD) is a gluten-sensitive enteropathy due to intolerance to dietary wheat gliadin and related proteins in genetically predisposed individuals[6]. The malabsorption of folates and vitamins (the deficiency of which may be a cause of hyperhomocysteinemia) is frequent in CD patients, either in the classic or oligosymptomatic type[7], and several cases of thrombosis have been reported in patients with CD before establishing a diagnosis of gluten-related duodenal mucosal damage[8,9]. Thus, we investigated the presence of hyperhomocysteinemia in a series of patients with CD, to see whether it might be increased and represent a marker of increased venous thrombosis in these patients.

MATERIALS AND METHODS

Patients

In the period January 1998-December 2008, 165 patients with CD (27 men, 138 women, mean age 43 years) were studied. Inclusion criteria were: positivity for anti-endomysial IgA and anti-transglutaminase IgA antibodies (Eurospital, Trieste, Italy) and duodenal histology suggestive for CD.

Histological assessment

Four samples were obtained by endoscopy forceps from the proximal and distal parts of the duodenum. The biopsies, correctly oriented on acetate cellulose filters (Bio-Optica, Milano, Italy), were fixed in 10% buffered formalin, processed and included in paraffin. After obtaining 5 μm thick sections, these were stained with Hematoxylin-Eosin; some sections were also processed for immunohistochemistry and stained with an anti-CD3 monoclonal antibody (Dako, Denmark) to identify intraepithelial lymphocytes (IEL). IEL density was expressed as the number of IEL/100 epithelial cells, with a density value of > 25 cells considered as pathological. Histological classification was based on the Marsh-Oberhuber criteria[10] and a new, recently proposed simpler classification[11,12] (Table 1).

Laboratory assessment

Serum homocysteinemia, vitamin B12 and folic acid levels were measured in all patients. In case of hyperhomocysteinemia, mutations in N5-N10-metil tetrahydrofolate reductase (MTHFR), cystathionine beta synthetase (CBS) and the prothrombin gene were searched for. DNA was extracted from whole blood collected in tubes containing K3-EDTA using a commercial kit (Genomic DNA Isolation kit, Puregene -Gentra System). DNA analysis for MTHFR gene mutation (C677T) was performed by a PCR-RFLP method, as previously described[13]. A fragment of 232 base pairs was then amplified by polymerase chain reaction. The fragment was digested by *Hin*f I restriction enzyme, and subsequent electrophoresis on ethidium bromide stained 3% agarose gel was performed.

The concentration of total homocysteine in plasma (K3-EDTA tubes) was determined by high performance liquid chromatography, as previously described[14]. Basal hyperhomocysteinemia (normal value 5-15 μmol/L) was classified as moderate (16-30 μmol/L), intermediate (31-100 μmol/L) and severe (> 100 μmol/L) according to Hankey *et al*[15]. In all patients, the presence of any thrombotic episode was also evaluated.

The study was approved by the Institutional Review Board of the Desio Hospital.

Table 1 The Marsh-Oberhuber classification of duodenal histological lesions in celiac disease, compared to the "simplified classification"[11,12]

Histologic type	IEL	Glandular crypts	Villi	Simplified
0	Normal (< 25/100 epithelial cells)	Normal	Normal	Normal
1	Increased	Normal	Normal	Grade A
2	Increased	Hyperplastic	Normal	Grade A
3a	Increased	Hyperplastic	Mild atrophy	Grade B1
3b	Increased	Hyperplastic	Moderate atrophy	Grade B1
3c	Increased	Hyperplastic	Severe (total) atrophy	Grade B2

IEL: Intra-epithelial lymphocytes.

RESULTS

Histological findings

Most CD patients (24/32, 75.0%) showed mild to severe villous atrophy, with the latter being present in 41.0% of patients (Table 2).

Laboratory findings

Overall, hyperhomocysteinemia was detected in 32 (19.4%) CD patients (24 women, 8 men); average symptoms' onset was 7 (range 1-40) years. Table 3 shows the serologic findings of these patients. Most patients (29/32, 91.0%) had moderate hyperhomocysteinemia, two (6.0%) intermediate and one (3.0%) severe increase of this value. Mutation of MTHFR was found in 13 (41.0%) patients, 7 homozygotes and 6 heterozygotes; one patient displayed heterozygotic mutation of the prothrombin gene. No CBS mutations were found.

Serum B12 vitamin levels were low in 5 (15.6%) patients and serum folate levels were low in 6 (19.0%) patients. No correlation (Spearman's test) was found between serum homocysteine and age ($r = 0.10$, $P = 0.58$), gender ($r = 0.66$, $P = 0.07$), onset of symptoms ($r = -0.06$, $P = 0.75$), vitamin B12 ($r = -026$, $P = 0.14$), folic acid ($r = 0.05$, $P = 0.75$), and histological grading ($r = -0.01$, $P = 0.9$). Moreover, no correlation was also found between histological grading, vitamin B12 ($r = -0.10$, $P = 0.56$) and folic acid ($r = -0.2$, $P = 0.3$) values.

Clinical findings

Concerning vascular pathology, one patient with heterozygosis for MTHFR mutation and moderate hyperhomocysteinemia had myocardial infarction, whereas the single

Table 2 Demographic, histological findings and associated diseases of 32 celiac disease patients with hyperhomocysteinemia

No.	Sex/age (yr)	Histology (Marsh/simplified classification)	Associated diseases
1	F/37	Marsh 2 (Grade A)	IgA deficit
2	F/38	Marsh 3a (Grade B1)	
3	M/34	Marsh 3a (Grade B1)	
4	F/32	Marsh 3c (Grade B2)	
5	F/20	Marsh 3c (Grade B2)	
6	F/47	Marsh 3b (Grade B1)	
7	F/64	Marsh 1 (Grade A)	
8	F/41	Marsh 3c (Grade B2)	Epilepsy
9	F/22	Marsh 2 (Grade A)	IgA deficit
10	F/61	Marsh 3a (Grade B1)	
11	M/44	Marsh 3c (Grade B2)	NASH
12	F/35	Marsh 3c (Grade B2)	type 1 diabetes
13	F/39	Marsh 3c (Grade B2)	
14	F/40	Marsh 3c (Grade B2)	
15	F/56	Marsh 3c (Grade B2)	PBC
16	F/35	Marsh 3c (Grade B2)	
17	F/42	Marsh 2 (Grade A)	
18	M/33	Marsh 3b (Grade B1)	Sarcoidosis
19	F/31	Marsh 3a (Grade B1)	
20	F/42	Marsh 3a (Grade B1)	
21	M/28	Marsh 3a (Grade B1)	
22	F/41	Marsh 3c (Grade B2)	
23	F/45	Marsh 3c (Grade B2)	
24	F/21	Marsh 3a (Grade B1)	
25	F/29	Marsh 3b (Grade B1)	
26	F/55	Marsh 3a (Grade B1)	Osteoporosis
27	F/78	Marsh 3c (Grade B2)	
28	M/47	Marsh 3c (Grade B2)	
29	M/63	Marsh 3b (Grade B1)	Psoriasis, myocardial infarction
30	M/18	Marsh 3a (Grade B1)	
31	M/40	Marsh 1 (Grade A)	
32	F/32	Marsh 3a (Grade B1)	Sarcoidosis

IgA: Immunoglobulin A; PBC: Primary biliary cirrhosis.

Table 3 Serologic findings of 32 celiac disease patients with hyperhomocysteinemia

No.	Serum homocysteine (NV 5-15 μmol/L)	Serum B12 vitamin (NV 190-66 pg/mL)	Serum folic acid (NV 2-14 ng/mL)	Genetic mutation
1	14	365	10	
2	13.5	267	4.7	
3	15	369	6	
4	20	356	1	
5	13	174	5	MTHFR (het)
6	21	311	1.5	
7	15	354	12	
8	44	293	5.2	MTHFR (hom)
9	15	493	6	MTHFR (hom)
10	17	333	1	MTHFR (het)
11	19	383	3	
12	17	198	2	
13	15	400	3	MTHFR (het)
14	27	720	4	MTHFR (hom)
15	13	699	3	MTHFR (hom)
16	21	246	2.4	
17	18	265	5.1	
18	19	282	1	Prothr (het)
19	20	457	3	MTHFR (hom)
20	14	555	0.5	
21	13	291	2	
22	23	280	6	
23	14	329	1	MTHFR (het)
24	17	684	10	
25	19	566	5.6	
26	20	188	2	
27	20.5	154	3	
28	20.5	216	2	MTHFR(het)
29	16	164	13	MTHFR (het)
30	25	555	8	
31	149	150	2	MTHFR (hom)
32	31	385	2	MTHFR (hom)

het: Heterozygosis; hom: Homozygosis; MTHFR: N5-N10-metil tetrahydrofolate reductase.

patient with severe hyperhomocysteinemia underwent coronary angiography for atypical chest pain, but no evidence of vessel pathology was found. No patient in this series had episodes of venous or arterial thrombosis, or any stroke episodes.

DISCUSSION

Our findings show that hyperhomocysteinemia is relatively frequent in patients with CD, being present in about 20% of the patients in our series. Hyperhomocysteinemia might represent a link between undiagnosed gluten-sensitive enteropathy and some of its complications[16]. Interestingly, these results were similar to those obtained in an overlapping geographic area, which showed the presence of hyperhomocysteinemia in about 20% of newly diagnosed CD patients compared to about 6% of controls[17].

Hyperhomocysteinemia may be due to genetic factors, with CBS deficiency being considered the most common genetic cause[5,17], or from acquired folate and vitamin B12 deficiencies[18,19]. A homozygous deficiency of MTHFR, the vitamin B12 dependent enzyme for the remethylation of homocysteine to methionine, may cause hyperhomocysteinemia and it has a worse prognosis than CBS deficiency for the absence of an effective therapy[20]. Moreover, treatment with a gluten-free diet and folic acid in CD patients with MTHFR variants does not consistently improve hyperhomocysteinemia[21].

Thus, CD (in which malabsorption of folate and vitamin B12 is common[22]) might lead to increased cardiovascular risks due to an increase of secondary (acquired) hyperhomocysteinemia, further aggravated by the possible presence of genetic abnormalities responsible for hyperhomocysteinemia. However, notwithstanding the relative frequency of hyperhomocysteinemia in our CD patients, this was almost always of moderate entity, with only one patient displaying high levels. Interestingly, the only patient to have a cardiovascular event (myocardial infarction) had relatively low levels of hyperhomocysteinemia and presented heterozygous mutations of MTHFR. No CBS mutations were found in our series. Only one mutation of the prothrombin gene was found, and this is in line with the paucity of reports of such mutations in CD patients[23].

In conclusion, at present it seems unnecessary to systematically investigate CD for the presence of hyperhomocysteinemia; conversely, a serological screening for CD in patients with hyperhomocysteinemia, cardiovascular events and vitamin deficiency could be considered, especially because adult CD patients may display only a few to no intestinal symptoms[24,25], and the onset of the disease may rarely be due to a thrombotic event[26-28].

COMMENTS

Background

Venous thrombosis has been reported in patients with celiac disease (CD). Since this might be related to hyperhomocysteinemia, a risk factor for vascular disease, we investigated the prevalence of hyperhomocysteinemia in a series of adult celiac patients.

Research frontiers

An increased prevalence of hyperhomocysteinemia in CD might lead to increased cardiovascular risk.

Innovations and breakthroughs

To date, most data on this topic originates from single reports, and only one other study investigated systematically celiac patients.

Applications

It appears that, given the low prevalence of hyperhomocysteinemia in celiac patients, it is unnecessary to screen systematically patients; this is useful information in terms of sanitary expenses.

Peer review

The authors evaluated in a cohort of 165 CD patients the presence of hyperhomocysteinemia during a period of time of 10 years. They showed that seems unnecessary to investigate systematically CD for the presence of hyperhomocysteinemia. Their work could contribute to the epidemiologic information of the CD in the Italian population.

REFERENCES

1 **Nygård O**, Vollset SE, Refsum H, Brattström L, Ueland PM. Total homocysteine and cardiovascular disease. *J Intern Med* 1999; **246**: 425-454
2 **Simioni P**, Prandoni P, Burlina A, Tormene D, Sardella C, Ferrari V, Benedetti L, Girolami A. Hyperhomocysteinemia and deep-vein thrombosis. A case-control study. *Thromb Haemost* 1996; **76**: 883-886
3 **den Heijer M**, Koster T, Blom HJ, Bos GM, Briet E, Reitsma PH, Vandenbroucke JP, Rosendaal FR. Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. *N Engl J Med* 1996; **334**: 759-762
4 **Welch GN**, Loscalzo J. Homocysteine and atherothrombosis. *N Engl J Med* 1998; **338**: 1042-1050
5 **Lim PO**, Tzemos N, Farquharson CA, Anderson JE, Deegan P, MacWalter RS, Struthers AD, MacDonald TM. Reversible hypertension following coeliac disease treatment: the role of moderate hyperhomocysteinaemia and vascular endothelial dysfunction. *J Hum Hypertens* 2002; **16**: 411-415
6 **Armstrong MJ**, Robins GG, Howdle PD. Recent advances in coeliac disease. *Curr Opin Gastroenterol* 2009; **25**: 100-109
7 **Bai JC**. Malabsorption syndromes. *Digestion* 1998; **59**: 530-546
8 **Hida M**, Erreimi N, Ettair S, Mouane N, Bouchta F. [Associated celiac disease and venous thrombosis]. *Arch Pediatr* 2000; **7**: 215-216
9 **Gabrielli M**, Santoliquido A, Gasbarrini G, Pola P, Gasbarrini A. Latent coeliac disease, hyperhomocysteinemia and pulmonary thromboembolism: a close link? *Thromb Haemost* 2003; **89**: 203-204
10 **Oberhuber G**, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. *Eur J Gastroenterol Hepatol* 1999; **11**: 1185-1194
11 **Corazza GR**, Villanacci V. Coeliac disease. *J Clin Pathol* 2005; **58**: 573-574
12 **Corazza GR**, Villanacci V, Zambelli C, Milione M, Luinetti O, Vindigni C, Chioda C, Albarello L, Bartolini D, Donato F. Comparison of the interobserver reproducibility with different histologic criteria used in celiac disease. *Clin Gastroenterol Hepatol* 2007; **5**: 838-843
13 **Kluijtmans LA**, van den Heuvel LP, Boers GH, Frosst P, Stevens EM, van Oost BA, den Heijer M, Trijbels FJ, Rozen R, Blom HJ. Molecular genetic analysis in mild hyperhomocysteinemia: a common mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for cardiovascular disease. *Am J Hum Genet* 1996; **58**: 35-41
14 **Accinni R**, Campolo J, Bartesaghi S, De Leo G, Lucarelli C, Cursano CF, Parodi O. High-performance liquid chromatographic determination of total plasma homocysteine with or without internal standards. *J Chromatogr A* 1998; **828**: 397-400
15 **Hankey GJ**, Eikelboom JW. Homocysteine and vascular disease. *Lancet* 1999; **354**: 407-413
16 **Saibeni S**, Lecchi A, Meucci G, Cattaneo M, Tagliabue L, Rondonotti E, Formenti S, De Franchis R, Vecchi M. Prevalence of hyperhomocysteinemia in adult gluten-sensitive enteropathy at diagnosis: role of B12, folate, and genetics. *Clin Gastroenterol Hepatol* 2005; **3**: 574-580
17 **Malinowska A**, Chmurzynska A. Polymorphism of genes encoding homocysteine metabolism-related enzymes and risk for cardiovascular disease. *Nutr Res* 2009; **29**: 685-695
18 **Cravo ML**, Glória LM, Selhub J, Nadeau MR, Camilo ME, Resende MP, Cardoso JN, Leitão CN, Mira FC. Hyperhomocysteinemia in chronic alcoholism: correlation with folate, vitamin B-12, and vitamin B-6 status. *Am J Clin Nutr* 1996; **63**: 220-224
19 **Cravo ML**, Camilo ME. Hyperhomocysteinemia in chronic alcoholism: relations to folic acid and vitamins B(6) and B(12) status. *Nutrition* 2000; **16**: 296-302
20 **Mudd SH**, Levy HL, Skovby F. Disorders of transsulfuration. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. The Metabolic and Molecular Basis of Inherited Disease. 7th ed. New York, NY: McGraw-Hill, 1995: 1279-1327
21 **Wilcox GM**, Mattia AR. Celiac sprue, hyperhomocysteinemia, and MTHFR gene variants. *J Clin Gastroenterol* 2006; **40**: 596-601
22 **Halfdanarson TR**, Litzow MR, Murray JA. Hematologic manifestations of celiac disease. *Blood* 2007; **109**: 412-421
23 **Gould J**, Deam S, Dolan G. Prothrombin 20210A polymorphism and third generation oral contraceptives--a case report of coeliac axis thrombosis and splenic infarction. *Thromb Haemost* 1998; **79**: 1214-1215
24 **Setty M**, Hormaza L, Guandalini S. Celiac disease: risk assessment, diagnosis, and monitoring. *Mol Diagn Ther* 2008; **12**: 289-298
25 **Rubio-Tapia A**, Murray JA. Celiac disease. *Curr Opin Gastroenterol* 2010; **26**: 116-122
26 **Kremer Hovinga JA**, Baerlocher G, Wuillemin WA, Solenthaler M. [Deep venous thrombosis of the leg in acquired thrombophilia--hyperhomocysteinemia as a sequela of undetected celiac disease]. *Ther Umsch* 1999; **56**: 519-522
27 **Audia S**, Duchêne C, Samson M, Muller G, Bielefeld P, Ricolfi F, Giroud M, Besancenot JF. [Stroke in young adults with celiac disease]. *Rev Med Interne* 2008; **29**: 228-231
28 **Baryshnikov EN**, Krums LM, Vorob'eva NN, Parfenov AI. [Lower extremity deep vein thrombosis associated with gluten-sensitivity celiac disease]. *Ter Arkh* 2010; **82**: 52-54

S- Editor Tian L **L- Editor** Rutherford A **E- Editor** Zheng XM