Is hyperhomocysteinemia relevant in patients with celiac disease?

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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.

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Key words: Celiac disease; Endoscopy; Histology; Hyperhomocysteinemia

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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.

Celiac disease (CD) is a gluten-sensitive enteropathy due to intolerance to dietary wheat gliadin and related proteins in genetically predisposed individuals. The malabsorption of folic acids and vitamins (the deficiency of which may be a cause of hyperhomocysteinemia) is frequent in CD patients, either in the classic or oligosymptomatic type, and several cases of thrombosis have been reported in patients with CD before establishing a diagnosis of gluten-related duodenal mucosal damage. 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 (Eurispital, 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 considered as pathological. Histological classification was based on the Marsh-Oberhuber criteria and a new, recently proposed simpler classification.

**Laboratory assessment**
Serum homocysteine, vitamin B12 and folic acid levels were measured in all patients. In case of hyperhomocysteinemia, mutations in N5-N10-methyl 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. A fragment of 232 base pairs was then amplified by polymerase chain reaction. The fragment was digested by Hinf 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. 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. 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.

**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 = -0.26, 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
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[20]. 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[21].

Hyperhomocysteinemia may be due to genetic factors, with CBS deficiency being considered the most common genetic cause[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, further aggravated by the possible presence of genetic abnormalities responsible for 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
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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 19 262 1 Proth (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 20 198 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 tetrahydrololate reductase.

**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)                         | type 1 diabetes     |
| 12  | F/35        | Marsh 3c (Grade B2)                         |                     |
| 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  | F/33        | Marsh 3b (Grade B1)                         | Sarcoïdosis         |
| 19  | F/31        | Marsh 3a (Grade B1)                         |                     |
| 20  | F/42        | Marsh 3a (Grade B1)                         |                     |
| 21  | F/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, myocar-  |
|    |             |                                             | dial infarction     |
| 30  | M/18        | Marsh 3a (Grade B1)                         |                     |
| 31  | M/40        | Marsh 1 (Grade A)                           |                     |
| 32  | F/32        | Marsh 3c (Grade B2)                         | Sarcoïdosis         |

IgA: Immunoglobulin A; PBC: Primary biliary cirrhosis.

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**Table 3** Serologic findings of 32 celiac disease patients with hyperhomocysteinemia

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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 symptoms24,25, and the onset of the disease may rarely be due to a thrombotic event26-28.

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. 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, Briel 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, Locasalo J. Homocysteine and atherothrombosis. N Engl J Med 1998; 338: 1042-1050
5 Lim PO, Tzemos N, Farquharson CA, Anderson JE, Deegan P, MacWhirter RS, Struthers AD, MacDonald TM. Reversible hypertension following coeliac disease treatment: the role of moderate hyperhomocysteinemia and vascular endothelial dysfunction. J Hum Hypertens 2002; 16: 411-415
6 Armstrong MJ, Robins CG, 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, Santoliquido A, Gabarrini G, Pola P, Gabarrini A. Latent coeliac disease, hyperhomocysteinemia and pulmonary thromboembolism: a close link? Thromb Haemost 2003; 89: 203-204
9 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: 1185-1189
10 Corazza GR, Villanacci V. Coeliac disease. J Clin Pathol 2005; 58: 573-574
11 Corazza GR, Villanacci V, Zambelli C, Milione M, Luietti O, Vindigni C, Chioda L, Bartoloni D, Donateo F. Comparison of the interobserver reproducibility with different histologic criteria used in celiac disease. Clin Gastroenterol Hepatol 2007; 5: 838-843
12 Kluijtmans LA, van den Heuvel LP, Boers GH, Frospot P, Stevens EM, van Oost BA, den Heijer M, Tijhols FJ, Rozen R, Blom HJ. Molecular genetic analysis in mild hyperhomocysteinemia: a common mutation in the methylene tetrahydrofolate reductase gene is a genetic risk factor for cardiovascular disease. Am J Hum Genet 1996; 58: 35-41
13 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; 829: 397-400
14 Hankey GJ, Elkelboom JW. Homocysteine and vascular disease. Lancet 1999; 354: 407-413
15 Saiben Si, Lecchi A, Meucci G, Cattaneo L, Chelabue L, Rondonetti F, Cornetti 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
16 Malinowska A, Chmiuryńska A. Polymorphism of genes encoding homocysteine metabolism-related enzymes and risk for cardiovascular disease. Nutr Res 2009; 29: 685-695
17 Cravo ML, Glória LM, Selhub J, Nadeau MR, Camilo ME, Re-sende MP, Cardoso JN, Leitão CN, Mira FC. Hyperhomocysteinemia in chronic alcoholism: correlation with folate, vitamin B-12, and vitamin B-6 status. J Clin Nutr 1999; 63: 220-224
18 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
19 Mudd SH, Levy HL, Skovby F. Disorders of transsulfuration. In: Scrivener 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
20 Wilcox GM, Mattia AR. Celiac sprue, hyperhomocysteinemia, and MTHFR gene variants. J Clin Gastroenterol 2006; 40: 596-601
21 Halfdanarson TR, Litzow MR, Murray JA. Hematologic manifestations of celiac disease. Blood 2007; 109: 412-421
22 Gould J, Dean S, Dolan G. Prothrombin 20210A polymor-phism and third generation oral contraceptives—a case report of coeliac axis thrombosis and splenic infarction. Thromb Haemost 1998; 79: 1214-1215
23 Setty M, Hornaza L, Guandalini S. Celiac disease: risk assessment, diagnosis, and monitoring. Mol Diagn Ther 2008; 12: 289-298
24 Rubio-Tapia A, Murray J A. Celiac disease. Curr Opin Gastroenterol 2010; 26: 116-122
25 Kremer Hovinga JA, Baerlocher G, Wulliem SWA, Solenhaver M. [Deep venous thrombosis of the leg in acquired thrombophilia—hyperhomocysteinemia as a sequela of undetected celiac disease]. Ther Umsch 1999; 56: 519-522
26 Audia S, Duchêne C, Sanson M, Muller G, Bielefeld P, Ricolfi F, Giroud M, Besancon JF. [Stroke in young adults with celiac disease]. Rev Med Interne 2008; 29: 228-231
27 Baryshnikov EN, Kruma LM, Vorob’eva NN, Parfenov AL. [Lower extremity deep vein thrombosis associated with gluten-sensitivity celiac disease]. Ter Arkh 2010; 82: 52-54

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