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doi:10.3748/wjg.15.955

World J Gastroenterol 2009 February 28; 15(8): 955-960
World Journal of Gastroenterology ISSN 1007-9327

Effect of B vitamin supplementation on plasma homocysteine levels in celiac disease

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Received: November 3, 2008 Revised: November 29, 2008
Accepted: December 6, 2008
Published online: February 28, 2009

Abstract

AIM: To investigate the effect of vitamin supplements on homocysteine levels in patients with celiac disease.

METHODS: Vitamin B6, folate, vitamin B12, and fasting plasma homocysteine levels were measured in 51 consecutive adults with celiac disease [median (range) age 56 (18-63) years; 40% men, 26 (51%) had villous atrophy, and 25 (49%) used B-vitamin supplements] and 50 healthy control individuals matched for age and sex. Finally, the C677T polymorphism of 5,10-methylene tetrahydrofolate reductase (MTHFR) was evaluated in 46 patients with celiac disease and all control individuals.

RESULTS: Patients with celiac disease and using vitamin supplements had higher serum vitamin B6 (P = 0.003), folate (P < 0.001), and vitamin B12 (P = 0.012) levels than patients who did not or healthy controls (P = 0.035, P < 0.001, P = 0.007, for vitamin B6, folate, and vitamin B12, respectively). Lower plasma homocysteine levels were found in patients using vitamin supplements than in patients who did not (P = 0.001) or healthy controls (P = 0.003). However, vitamin B6 and folate, not vitamin B12, were significantly and independently associated with homocysteine levels. Twenty-four (48%) of 50 controls and 23 (50%) of 46 patients with celiac disease carried the MTHFR thermolabile variant T-allele (P = 0.89).

CONCLUSION: Homocysteine levels are dependent on Marsh classification and the regular use of B-vitamin supplements is effective in reduction of homocysteine levels in patients with celiac disease and should be considered in disease management.

Key words: Celiac disease; Homocysteine; Vitamin supplements

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Hadithi M, Mulder CJJ, Stam F, Azizi J, Crusius JBA, Peña AS, Stehouwer CDA, Smulders YM. Effect of B vitamin supplementation on plasma homocysteine levels in celiac disease. *World J Gastroenterol* 2009; 15(8): 955-960 Available from: URL: http://www.wjgnet.com/1007-9327/15/955.asp DOI: http://dx.doi.org/10.3748/wjg.15.955

INTRODUCTION

Numerous studies suggest that moderate hyperhomocysteinemia is an independent risk factor for atherothrombotic vascular disease[1,2] and recurrent venous thromboembolism[3]. Moderate elevations in total plasma homocysteine (tHcy) levels are commonly due to acquired deficiencies in B vitamin cofactors of homocysteine metabolism, including vitamin B6, folate, and vitamin B12[4]. Earlier trials have demonstrated that daily supplementation of folate or vitamin B12 could normalize the concentration of tHcy[5]. The American Heart Association has recommended screening for

hyperhomocysteinemia in patients with malnutrition and malabsorption syndromes[6].

Celiac disease is a typical example of a malabsorption syndrome conferring increased risk for various deficiency states, including folate and vitamin B12[7]. Even patients adhering to a strict gluten-free diet for years were prone to the development of various vitamin deficiency states, although they were in biopsy-proven remission[8]. Moreover, hyperhomocysteinemia is significantly more frequent in patients with newly diagnosed celiac disease than healthy controls and has been reported to improve after the institution of a gluten-free diet[9,10].

In view of these considerations, we investigated the effect of vitamin B6, folate, and vitamin B12 daily supplements on tHcy levels in patients with celiac disease.

MATERIALS AND METHODS

Between March, 2004 and November, 2005, 51 consecutive celiac disease patients attending a clinic for either initial or follow-up small intestinal biopsy (at least 12 mo after commencing a gluten-free diet) were included in the study. Patients were diagnosed with celiac disease based on the European Society of Pediatric Gastroenterology, Hepatology and Nutrition diagnostic criteria[11]. All patients carried either HLA-DQ2 (encoded by DQA1*0501 and DQB1*02 alleles) or HLA-DQ8 (encoded by DQA1*0301 and DQB1*0302 alleles) or both.

Patients were categorized into four groups: (1) newly diagnosed (untreated; all positive to IgA anti-transglutaminase and to IgA anti-endomysium antibodies and all had villous atrophy; n = 7); (2) persistent villous atrophy at follow-up due to dietary mistakes (non-compliant; all were positive to either IgA anti-transglutaminase or to IgA anti-endomysium antibodies; n = 7); (3) persistent villous atrophy at follow-up despite strict adherence to gluten free diet for at least 12 mo (refractory; all were negative to both serum antibody tests; n = 12); or (4) recovered villous architecture at follow-up biopsy (responsive to gluten free diet; six were positive to either IgA anti-transglutaminase or to IgA anti-endomysium antibodies; n = 25).

A dedicated dietician assessed the dietary behavior of all patients with celiac disease. Overall, twenty-five (49%) patients with celiac disease reported the regular daily use of vitamin supplements containing vitamin B6 (range 1-6 mg), folate (range 100-400 μg), and vitamin B12 (range 0.5-18 μg) for a median interval of 28 mo (range 18-84).

Blood samples were analyzed for serum vitamin B6, folate, vitamin B12, and fasting tHcy levels in all study patients within a median of 16 d (range 7-30 d) from small bowel biopsy.

In addition, serum vitamins and tHcy were measured in 50 healthy individuals, matched for age and sex, who served as controls. No individuals in the control group reported the use of vitamin supplementation. No patients with celiac disease or from the control group reported the use of drugs known to affect tHcy levels (e.g. diphantoine, methotrexate or theophylline). Finally, the C677T polymorphism of 5, 10-methylenetetrahydrofolate reductase (MTHFR) was evaluated in 46 patients with celiac disease and all controls. All controls and 90% of the patients with celiac disease were whites of European descent.

The Ethics Committee of the VU University Medical Center approved the study protocol, and all participants gave their written informed consent.

Laboratory analyses

Microparticle enzyme immunoassay method based on fluorescence polarization (IMX analyzer; Abbott, Chicago, IL, U.S.A.) was used to measure tHcy. Intra- and inter-assay CVs (coefficients of variation) were 2.1 and 5.1%, respectively[12]. Serum creatinine was measured by means of a modified Jaffé method and creatinine clearance was calculated according to the Cockroft-Gault method. Serum vitamin B6, folate, and vitamin B12 were measured with a competitive protein-binding assay (Dualcount Solid Phase Boil assay, DPC, Los Angeles, CA, USA; reference values 10-50 nmol/L, 6.8-39 nmol/L, and 150-700 pmol/L, respectively).

Genotyping of the MTHFR C677T SNP by polymerase chain reaction

A procedure using the commercial DNAzol reagent was applied to extract genomic DNA from peripheral blood. The region surrounding the MTHFR C677T SNP (dbSNP ID: rs1801133) was amplified using the polymerase chain reaction (with the primers 5'-TGAAGGAGAAGGTGTCTGCGGGA-3' and 5'-AGGACGGTGCGGTGAGAGTG-3') as originally described[13]. PCR conditions were: 95℃ for 2 min, followed by: 35 cycles of 95℃ for 30 s, 55℃ for 30 s, 72℃ for 30 s, followed by a final extension of 5 min at 72℃. Since the MTHFR C677T SNP creates a restriction site for *Taq* I the 198 bp amplified fragment was digested by *Taq* I , followed by agarose gel electrophoresis and ethidium bromide staining, resulting in a 198 bp fragment when allele C is present and fragments of 171 bp and 27 bp when allele T is present.

Statistical analysis

Patients with celiac disease were once stratified into two subgroups with respect to the use of vitamin supplements [confirmed in 25 (49%) patients] and at another time into another two subgroups with respect to the presence of villous atrophy [documented in 26 (51%) patients]. The results were analyzed and compared between the subgroups and the controls. Continuous data having normal distribution are presented as mean (SD), and skewed data are presented as median (interquartile range). Categorical data are presented in frequencies and percentages. Comparison between groups was performed by means of the two-tailed t tests for data with normal distribution and the Mann-Whitney-U or Kruskal-Wallis tests when a non-parametric test was indicated. Pearson χ^2 or

Fisher's exact test when appropriate was used to compare categorical data. Independent associations between age, creatinine clearance, the carriage of MTHFR mutation, serum B6, folate, and B12 with tHcy were assessed by means of multiple linear regression analysis. For all statistical analyses, $P < 0.05$ was considered significant. All statistical tests were performed using the Statistical Software Package version 11.0 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Baseline clinical and biochemical characteristics of patients with celiac disease and controls are presented in Table 1. Patients with celiac disease had lower body mass index and creatinine clearance than healthy controls. When patients with celiac disease were stratified into subgroups according to their regular vitamin use, no differences appeared with regards to the body mass index or creatinine clearance (data not shown). The celiac-specific serological tests were also comparable between patients with celiac disease who did and who did not use vitamin supplements.

The median serum vitamin B6, folate, and vitamin B12 levels were significantly higher in patients with celiac disease on vitamin supplements compared to patients not using vitamin supplements and to healthy controls (Table 2). In accordance, patients with celiac disease taking vitamins had lower tHcy levels than patients who did not use vitamins and healthy controls ($P = 0.001$ and $P = 0.003$, respectively). Despite the absence of significant differences in B6, folate, and B12 status, tHcy was marginally, but significantly higher in celiac disease patients not using vitamins compared to controls ($P = 0.04$).

When patients with celiac disease were stratified into two groups according to small bowel histology, villous atrophy was found to be associated with higher tHcy levels ($P = 0.018$) while vitamin B6, folate, and vitamin B12 levels were comparable (Table 3). The finding was also accounted for by the celiac disease group not using vitamins ($P = 0.04$), but not for those using vitamins ($P = 0.2$).

Twenty-four (48%) of 50 controls and 23 (50%) of 46 patients with celiac disease carried the MTHFR thermolabile variant T-allele ($P = 0.89$). Genotype data were in Hardy-Weinberg equilibrium for the control subjects ($P > 0.05$). Individuals homozygous for the MTHFR C>T mutation was more frequently observed among controls than among patients with celiac disease (14% *vs* 7%; $P = 0.23$).

Multiple regression analyses showed that vitamin B6 and folate, but not vitamin B12, were significantly and independently associated with tHcy. Additional analyses revealed that the presence of celiac disease was associated with high tHcy levels independent of vitamin B6 or folate (Table 4). Additional adjustments for creatinine clearance, age, and MTHFR genotype did not affect this result (data not shown). As these results suggested that celiac disease affects tHcy independent of measured determinants of homocysteine, we analyzed the independent association with the severity of celiac disease, as assessed by the Marsh classification, and adjusted for vitamin B6 and folate. This analysis confirmed that the Marsh classification was an independent determinant of tHcy (Table 4). Again, additional adjustment for creatinine clearance, age, and MTHFR genotype did not affect the result.

Table 1 Baseline characteristics of patients with celiac disease and healthy controls

Characteristic	Healthy controls (n = 50)	Celiac disease (n =51)	P[1]
Median age (range), yr	48 (18-62)	56 (18-63)	0.09
Men, n (%)	22 (44%)	20 (39%)	0.7
Mean body mass index ± SD	25.0 ± 3.6	22.0 ± 2.7	< 0.001
Smoking, n (%)	4 (8.0%)	4 (7.8%)	1.0
Median creatinine clearance (range), mL/min	89.85 (54-124)	76.2 (40-156)	< 0.001

[1]Continuous data were compared by Student's t test (two-sided) and categorical data were compared by Fisher's exact test (two-sided).

DISCUSSION

This study demonstrated that patients with celiac disease using B vitamin supplements had lower tHcy levels than those who did not use B vitamin supplements. Second, even if villous atrophy persists, B-vitamin supplements can normalize B6, folate, B12 status, and tHcy levels. Finally, both the presence and the severity of celiac disease were determinants of tHcy levels, independent of measured B-vitamin status.

Serum B vitamins are essential cofactors in the metabolism of homocysteine, and vitamin B supplements can lower tHcy levels[14]. This finding is confirmed in our data from celiac disease patients. Patients with celiac disease not using vitamin supplements had higher tHcy levels than healthy controls despite comparable vitamin B6, folate, and B12 levels, and the presence of celiac disease as such was found to be a determinant of tHcy levels, independent of B6, folate, and B12 status. One explanation for this finding might be that tHcy is a more sensitive indicator of subtle B-vitamin deficiency compared to plasma B vitamin levels. Alternatively, unknown mechanisms related to celiac disease or variables not measured in this study, like riboflavin[15] or serine status[16], might play a role. A plausible explanation for the lower creatinine clearance in the celiac group might be the age factor, although this was not significantly different ($P = 0.09$). Creatinine clearance, however, did not appear to correlate with tHcy levels by further analysis.

Previous studies addressing the issue of B vitamin status and hyperhomocysteinemia in celiac disease have found similar results. In a study of 30 adult patients with celiac disease, tHcy levels were found raised in comparison with the general population[8]. In a prospective study, Saibeni *et al*[9] confirmed that hyperhomocysteinemia was significantly more frequent in patients newly diagnosed with celiac disease than

Table 2 Serum vitamin B6, folate, vitamin B12 and plasma homocysteine levels in patients with celiac disease (without and with regular vitamin supplements) and in healthy controls

Variable	Healthy controls (n = 50)	Patients with celiac disease not using vitamins (n = 26)	Patients with celiac disease using vitamins (n = 25)	P[1]
Median vitamin B6 (interquartile range), nmol/L	39.0 (30.2-54.2)	36.0 (21.0-77.2)	74.0 (28.0-183.5)	0.016
Median folate (interquartile range), nmol/L	9.7 (7.2-12.5)	12.1 (7.2-16.5)	29.9 (14.9-57.0)	< 0.001
Median vitamin B12 (interquartile range), pmol/L	234.5 (190.0-277.5)	230.5 (176.5-299.0)	342.0 (208.0-536.5)	0.017
Median homocysteine (interquartile range), μmol/L	9.7 (8.4-11.9)	11.0 (9.2-14.3)	7.1 (6.2-10.7)	0.001
Villous atrophy, n %		14 (54.0%)	12 (48.0%)	0.78

[1]Continuous data were compared by Kruskall-Wallis test and categorical data were compared by Fisher's exact test (two-sided).

Table 3 Serum vitamin B6, folate, vitamin B12 and plasma homocysteine levels in patients with celiac disease without (Marsh 0-Ⅱ) and with (Marsh Ⅲ) villous atrophy

Variable	All patients with celiac disease (n = 51)			Patients with celiac disease not using vitamins (n = 26)			Patients with celiac disease using vitamins (n = 25)		
	Marsh Ⅲ (n = 26)	Marsh 0-Ⅱ (n = 25)	P value[1]	Marsh Ⅲ (n = 14)	Marsh 0-Ⅱ (n = 12)	P value[1]	Marsh Ⅲ (n = 12)	Marsh 0-Ⅱ (n = 13)	P value[1]
Median vitamin B6 (interquartile range), nmol/L	54.5 (20.7-99.7)	60.0 (33.0-170.5)	0.26	34.0 (20.7-71.2)	61.5 (23.5-96.5)	0.59	72.0 (17.2-165.0)	91.0 (45.0-238.0)	0.47
Median folate (interquartile range), nmol/L	14.2 (7.2-33.9)	19.8 (11.5-28.9)	0.41	12.2 (6.2-16.1)	12.1 (7.7-19.1)	0.49	34.8 (11.7-75.4)	27.8 (18.6-36.1)	0.93
Median vitamin B12 (interquartile range), pmol/L	263.0 (195.2-433.0)	278.0 (177.5-353.5)	0.45	229.0 (173.7-295.5)	254.5 (171.7-303.0)	0.52	455.5 (256.7-916.2)	317.0 (180.0-380.0)	0.04
Median homocysteine (interquartile range), μmol/L	11.0 (8.1-16.1)	9.1 (6.7-10.7)	0.018	12.6 (10.5-17.4)	9.3 (9.1-13.4)	0.04	8.9 (6.1-13.2)	7.1 (6.1-8.8)	0.20
Use of vitamin supplements, n%	12 (46.1%)	13 (52.0%)	0.78[2]						

[1]Continuous data were compared by Kruskall-Wallis test; [2]Vitamin use versus no vitamin use frequency was compared by Fisher's exact test (two-sided).

in healthy controls[9]. Dickey *et al*[10] described the homocysteine and biomarker status of metabolically related B vitamins in patients with celiac disease (newly diagnosed = 35, non-responsive despite gluten free diet = 24, and recovered after gluten free diet = 41) and found that gluten exclusion in celiac disease improved folate status and normalized homocysteine levels. Our study demonstrates, in agreement with previous reports, that celiac disease is associated with elevated tHcy levels.

High tHcy concentrations in individuals with thermolabile MTHFR (T allele) have been reported among those patients with reduced plasma folate concentrations[17-19]. However, we found no significant differences when these two groups were compared (data not shown).

The consequences of higher tHcy levels in celiac disease may include an increased tendency to develop occlusive venous and arterial disease. Although this has been an understudied area, data are emerging that celiac disease confers an increased risk of vascular complications. The association of hepatic vein obstruction and celiac disease, for example, has been reported previously in five children[20] and in three adults[21]. Case reports have repeatedly described the concomitant presence of celiac disease, hyperhomocysteinemia, and thromboembolic events, like deep vein thrombosis[22], stroke[23], or pulmonary embolism[24]. The consequences of long-term (subtle) B vitamin deficiency may result in an increased propensity to develop malignancy[25], including lymphoma[26].

Table 4 The independent association of homocysteine with vitamin B6, folate, the presence of celiac disease, and villous atrophy, analyzed by multiple regression analysis

	Standardized coefficients beta	Significance
Presence of celiac disease		
Vitamin B6	-0.309	0.002
Folate	-0.366	0.000
Celiac disease	-0.194	0.053
Presence of villous atrophy		
Vitamin B6	-0.307	0.013
Folate	-0.363	0.004
Villous atrophy	0.294	0.018

Dependent variable: Homocysteine.

With respect to the effect of a gluten free diet, a previous study revealed the presence of elevated tHcy levels in patients with celiac disease on strict gluten free diet for ten years[8]. Others, however, have found that gluten free diet was able to normalize hyperhomocysteine levels[9,10].

Patients adhering to a strict gluten free diet are still prone to the development of various vitamin deficiency states[8]. Earlier, thiamin, riboflavin, and niacin contents of gluten free cereal products have been compared with their gluten containing counterparts[27]. Most gluten free

food has been found to provide lower amounts of at least one of these nutrients. In another report, 30 of 37 gluten free cereal products with available data on folate content contained lower amounts of folate compared with their gluten containing counterparts[28]. Of the 58 gluten free breads, pastas, and cold cereals, only 3 cold cereals were fortified with folic acid. None of the bread products or pastas were enriched with folic acid[28]. Consequently, the use of vitamin supplements next to gluten free diet in patients with long standing celiac disease has been advocated[8]. The favorable effect of vitamin supplements on B-vitamin and tHcy levels has been previously established and clearly appears to apply to patients with celiac disease[29,30].

Limitations of this study include the heterogeneity of study subjects that considerably affected the study design. Second, B-vitamin supplement treatment was not standardized. Third, the lack of follow-up data in our study did not allow us to reach conclusions regarding the effect of gluten free diet alone on tHcy levels. The recovery of villous atrophy following a gluten free diet has been earlier shown to normalize homocysteine levels[10]. However, treatment with a gluten-free diet and folic acid in case studies led to the variable improvement in homocysteine levels[31]. A useful study would be to randomize newly diagnosed patients with celiac disease to use B-vitamin supplements with a gluten free diet compared to a gluten free diet alone. Finally, it might be argued that current evidence suggests that there is no clinical benefit of the use of folic acid and vitamin B12 (with or without addition of vitamin B6) in patients with established vascular disease[32]. However, many other studies conducted over the past 25 years have provided ample support to the association of mild hyperhomocysteinemia with an elevated risk of atherosclerosis. In addition, several issues remain unresolved and require further investigation, including the proper dose of B-vitamin supplements, the proper duration of treatment, the timing of treatment (i.e. in primary or secondary prevention setting), and the implications of lower rates of stroke[33]. In any case, long-term B vitamin deficiency, which is clearly a risk associated with celiac disease, should be avoided and thus requires either monitoring of B vitamin levels and tHcy or standard treatment with moderate-dose B vitamin supplements.

In conclusion, celiac disease, the presence of villous atrophy, vitamin B6 and folate are independent determinants of tHcy levels. Use of B-vitamin supplements lowers tHcy levels, even if villous atrophy persists.

COMMENTS

Background

Celiac disease increases the risk for folate and vitamin B12 deficiency. Consequently, this can contribute to the development of hyperhomocysteinemia with its associated link to atherothrombotic vascular disease.

Innovations and breakthroughs

Although a gluten free diet can reverse deficiency states that can develop in patients with celiac disease, vitamin supplements have been shown to have an additional value in this context. In this study, the authors demonstrate that B-vitamin supplements to a gluten free diet has a protective role against the development of hyperhomocysteinemia, even when villous atrophy did not recover yet.

Applications

The regular use of B-vitamin supplements reduces the risk of hyperhomocysteinemia in patients with celiac disease and should be considered in daily clinical management.

Terminology

Hyperhomocysteinemia can develop secondary to B-vitamin deficiency states, which is likely to occur in patients with celiac disease. Not surprisingly, B-vitamin supplements contribute to the reversal of hyperhomocysteinemia in patients with celiac disease.

Peer review

This study contributes to what is already known about homocysteine status in celiac disease.

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