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• *BRIEF REPORTS* •

Helicobacter pylori eradication lowers serum homocysteine level in patients without gastric atrophy

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Abstract

AIM: To determine whether *Helicobacter pylori* (*H pylori*) infection caused hyperhomocysteinemia by altering serum vitamin B_{12}, serum folate and erythrocyte folate levels and whether eradication of this organism decreased serum homocysteine level.

METHODS: The study involved 73 dyspeptic *H pylori*-positive patients, none of them had gastric mucosal atrophy based on rapid urease test and histology. Out of 73 patients, 41 (56.2%) showed a successful eradication of *H pylori* 4 wk after the end of treatment. In these 41 patients, fasting serum vitamin B_{12}, folate and homocysteine levels, and erythrocyte folate levels before and 4 wk after *H pylori* eradication therapy were compared.

RESULTS: The group with a successful eradication of *H pylori* had significantly higher serum vitamin B_{12} and erythrocyte folate levels in the post-treatment period compared to those in pre-treatment period (210±97 pg/mL *vs* 237±94 pg/mL, $P<0.001$ and 442±212 ng/mL *vs* 539±304 ng/mL, $P = 0.024$, respectively), but showed no significant change in serum folate levels (5.6±2.6 ng/mL *vs* 6.0±2.4 ng/mL, $P = 0.341$). Also, the serum homocysteine levels in this group were significantly lower after therapy (13.1±5.2 μmol/L *vs* 11.9±6.2 μmol/L, $P = 0.002$). Regression analysis showed that serum homocysteine level was positively correlated with age ($P = 0.01$) and negatively with serum folate level before therapy ($P = 0.003$).

CONCLUSION: Eradication of *H pylori* decreases serum homocysteine even in patients who do not exhibit gastric mucosal atrophy. It appears that the level of homocysteine in serum is related to a complex interaction among serum vitamin B_{12}, serum folate and erythrocyte folate levels.

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Key words: *H pylori*; Gastritis; Vitamin B_{12}; Folate; Erythrocyte folate; Homocysteine

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INTRODUCTION

Helicobacter pylori (*H pylori*) is a spiral-shaped bacterium that causes chronic infection in human stomachs, and often leads to gastritis and peptic ulcers[1]. Recent data indicate a possible correlation between *H pylori* infection and coronary heart disease[2,3]. The connection between *H pylori* infection and hyperhomocysteinemia is one way in which this organism may be linked to the development of coronary diseases. Researches have shown strong associations between hyperhomocysteinemia and inadequate vitamin intake and insufficient vitamin concentrations in plasma, particularly vitamin B_6, vitamin B_{12} and folate levels[4,5]. Several studies have demonstrated that *H pylori* infection has negative effects on serum levels of vitamin B_{12} and folate[6-8].

Homocysteine metabolism involves a complex interaction between folate and vitamin B_{12}[9]. Our aims in this study were to examine whether *H pylori* infection affected serum homocysteine, serum vitamin B_{12}, serum folate, and erythrocyte folate levels in non-ulcer dyspeptic patients without gastric mucosal atrophy, and to evaluate the effect of eradication of *H pylori* on serum homocysteine level.

MATERIALS AND METHODS

Patients

This study included 73 dyspeptic patients (24 men and 49 women; mean age 41±12 years) and was conducted between May 2002 and February 2003. The protocol was approved by the Human Research Ethics Committee of Baskent University, and informed consent was obtained from all subjects. Each individual was referred to our center for endoscopic examination, and diagnosed with *H pylori* infection by rapid urease test (Pronto Dry, Medical Instr., Solothurn, Switzerland) and histolopathologic evaluation. In each case, two biopsy specimens from the gastric antrum

and two from the corpus were examined. The tissues were stained with hematoxylin and eosin, and Giemsa stain. Gastritis was defined using the Sydney classification[10]. The same pathologist, who was blinded to the clinical conditions of the patients, performed all histological examinations. None of these dyspeptic patients exhibited gastric mucosal atrophy. All patients underwent *H pylori* eradication therapy (2 wk of a combination regimen of lansoprazole 30 mg twice daily, amoxicillin 1 000 mg twice daily, and clarithromycin 500 mg twice daily). Repeat endoscopy was done 4 wk after the completion of treatment to assess the eradication status of *H pylori* in each patient.

Before and at the time of the investigation, none of the 73 patients was taking medication known to alter serum homocysteine levels, such as methotrexate, theophylline, anticonvulsants or antidepressants. The other exclusion criteria were chronic renal failure, hypothyroidism, previous gastric surgery, smoking habit, and use of proton pump inhibitors, antibiotics or vitamin supplementation in the 4 wk prior to enrollment in the study.

Determination of serum homocysteine, vitamin B_{12}, folate, and erythrocyte folate levels

A blood sample was drawn from each patient before and 4 wk after the completion of eradication therapy. Each sample was collected after overnight fasting, and serum homocysteine, serum vitamin B_{12}, serum folate, and erythrocyte folate levels were measured. Serum homocysteine level was determined using a commercial fluorescence polarization immunoassay (AXSYM Homocysteine, Abbott Laboratories, Abbott Park, IL, reference ranges for male and females were 5.9-16.0 and 3.36-20.44 μmol/L, respectively). Serum vitamin B_{12} was measured by electrochemiluminescence immunoassay (Elecsys Vitamin B_{12}, Roche, IN, reference range: 197-866 pg/mL), and serum folate and erythrocyte folate levels were determined using a binding assay technique (Elecsys folate, Roche, IN, reference range: 3-17 and 93-641 ng/mL, respectively).

Table 1 Serum vitamin B_{12}, folate, erythrocyte folate, and homocysteine levels before and after *H pylori* eradication treatment (mean±SD)

	Patients with successful eradication (n = 41)		
	Before treatment	After treatment	P
Serum vitamin B_{12} (pg/mL)	210±97	237±94	<0.001
Serum folate (ng/mL)	5.6±2.6	6.0±2.4	0.341
Erythrocyte folate (ng/mL)	442±212	539±304	0.024
Serum homocysteine (μmol/L)	13.1±5.2	11.9±6.2	0.002

Table 2 Relationships between serum homocysteine level and serum or erythrocyte levels of vitamins, and patient age and sex before *H pylori* eradication

	Pearson's ρ	P
Homocysteine–age	0.272	0.020
Homocysteine–sex	–0.201	0.088
Homocysteine–B_{12}	–0.267	0.023
Homocysteine–serum folate	–0.367	0.001
Homocysteine–erythrocyte folate	–0.336	0.004
Serum folate–erythrocyte folate	0.654	<0.001

Statistical analysis

All analyses were performed using the statistical package for the social sciences (SPSS) for Windows, version 9.05. Normality of the distribution of the results for the four variables (serum vitamin B_{12}, serum folate, serum homocysteine, erythrocyte folate) were tested using the Kolmogorov-Smirnov test, and all were found to be normally distributed. Data were presented as mean±SD. P values <0.05 were considered statistically significant. The paired t-test was used to compare pre- and post-treatment serum levels of homocysteine, vitamin B_{12}, folate, and erythrocyte folate levels in all patients. Univariate analysis using Pearson's correlation test was done to evaluate the relationship between serum homocysteine and age, sex, serum folate, and erythrocyte folate levels. Logistic regression analysis was done to identify which parameters independently influenced serum homocysteine level. The independent variables tested in this model were age, sex, serum folate and vitamin B_{12} levels.

RESULTS

Of the 73 patients, 41(56.2%) showed a successful eradication of *H pylori* 4 wk after the end of treatment. The group with a successful eradication of *H pylori* had significantly higher serum vitamin B_{12} and erythrocyte folate levels in the post-treatment period compared to those in pre-treatment period (210±97 pg/mL *vs* 237±94 pg/mL, P<0.001 and 442±212 ng/mL *vs* 539±304 ng/mL, P = 0.024, respectively), but showed no significant change in serum folate levels (5.6±2.6 ng/mL *vs* 6.0±2.4 ng/mL, P = 0.341) (Table 1). Also, the serum homocysteine levels in this group were significantly lower after the therapy (13.1±5.2 μmol/L *vs* 11.9±6.2 μmol/L, P = 0.002) (Table 1 and Figure 1).

Univariate analysis of the data collected before eradication treatment revealed that serum homocysteine level was positively correlated with the age of the patient (r = 0.272; P = 0.02), and negatively with serum folate level (r = -0.367; P = 0.001), serum vitamin B_{12} level (r = -0.267; P = 0.023), and erythrocyte folate level (r = -0.336; P = 0.004) (Table 2). Regression analysis identified age (P = 0.01) and serum folate level (P = 0.003) as the only two factors independently associated with serum homocysteine level. The erythrocyte folate level, which was strongly correlated with serum folate level (Table 2), was not included in multivariate analysis.

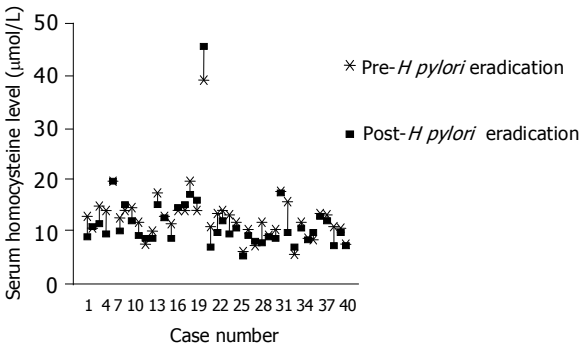

Figure 1 Changes in serum homocysteine level before and after *H pylori* eradication (P = 0.002).

DISCUSSION

Several recent studies have investigated the relationship between coronary artery disease and *H pylori* infection, and the results are controversial[2,3,11-13]. Hyperhomocysteinemia is a factor that is suggested to be responsible for the development of atherosclerosis in the setting of chronic *H pylori* infection. In recent years, homocysteine has been shown to be an important contributor to atherosclerosis[14-16]. One meta-analysis involving 10 000 patients revealed no meaningful correlations between *H pylori* and vascular risk factors[17]. Research has shown that homocysteine can directly cause endothelial damage[18], affect platelet function and coagulation factors[19], and increase the oxidation of low-density lipoproteins[20]. In the light of these findings, a number of investigators have focused on *H pylori* infection as a possible cause of hyperhomocysteinemia. However, these findings are also inconsistent[12,13,21,22].

It has been well established that chronic *H pylori* infection causes atrophic gastritis[1], and decreased absorption of both vitamin B_{12} and folic acid has been documented in patients with this condition[9]. Furthermore, a recent study done at our clinic showed that even patients with non-atrophic *H pylori* gastritis exhibited low vitamin B_{12} levels[8,23-25]. This is supported by investigations that demonstrated food-cobalamin malabsorption in patients with *H pylori* gastritis who did not have mucosal atrophy[8]. As noted above, in the present study we found that serum vitamin B_{12} level was significantly higher after treatment, regardless of the patient's eradication status. This indicates that the degree of malabsorption, or perhaps consumption of this vitamin by the organism, decreases when *H pylori* density in the gastric mucosa is reduced by eradication therapy. Patients with chronic *H pylori* infection exhibited decreased secretion of ascorbic acid by the gastric mucosa and elevated gastric pH[26,27]. It has been demonstrated that low levels of ascorbic acid in gastric juice or high pH of gastric juice could cause less folate absorption from the diet[28]. In the 41 dyspeptic *H pylori*-positive patients we studied, baseline serum folate levels were in the normal range and there was no significant change in this parameter after therapy (5.6±2.6 ng/mL *vs* 6.0±2.4 ng/mL, $P = 0.341$). We suspect that this may be because the levels were normal before treatment, and because the typical Turkish diet contains high levels of folate. Our patients also showed significantly higher erythrocyte folate levels after *H pylori* eradication therapy. This is also likely linked to vitamin B_{12} levels, since one important reaction in erythrocytes involves vitamin B_{12}-dependent transfer of the methyl group from N^5methylenetetrahydrofolate to homocysteine. Lack of adequate vitamin B_{12} impedes this reaction and leads to leakage of unconjugated folate from cells, whereas correction of the deficiency could restore erythrocyte folate levels[29].

Our analysis identified the age of the patient and serum folate level as independent determinants of serum homocysteine level. As described above, folate re-methylation of homocysteine to methionine required vitamin B_{12}. However, this vitamin had less influence on serum homocysteine concentration than serum folate[30]. Some authors have stressed that it is incorrect to state that vitamin B_{12} plays no role in homocysteine metabolism, and that the effect of this vitamin is often masked by the role of folate[31]. Also, research showed that when oral folic acid supplementation provided a certain serum level of folate (10 μg/L was considered the approximate cut-off), serum folate had less influence on homocysteine levels[31]. Our results are in line with these findings and statements. We conclude that serum folate level is a primary determinant of serum homocysteine level in dyspeptic *H pylori*-positive patients, even though infection with this organism is known to reduce serum B_{12} levels. Our study did not show that increased serum vitamin B_{12} after *H pylori* eradication had a positive effect on serum homocysteine levels, but like other authors, we believe that the impact of vitamin B_{12} may be indirect or masked.

One report in the literature states that each 1-μmol/L drop in serum homocysteine level represents a 10% decrease in the risk of vascular disease[14]. We found that the mean serum homocysteine level in our patients with complete *H pylori* eradication was decreased by slightly more than 1 μmol/L. Although homocysteine has not been considered to be as important as other risk factors such as hypercholesterolemia, smoking, diabetes mellitus, and hypertension, we suggest that prolonged hyperhomocysteinemia possibly due to *H pylori* infection since childhood, especially in developing countries may play a contributing role in the pathogenesis of atherosclerosis. This suggests that eradication of this microorganism can lower the risk of vascular diseases in dyspeptic *H pylori*-positive patients. It also indicates that there is a significant benefit to prescribing *H pylori* eradication even in the absence of mucosal atrophy or other severe gastroduodenal lesions. Such a treatment may be more important in countries where the rates of nutritional folic acid and/or vitamin B_{12} deficiency are particularly high. The property of diet consumed in a population is certainly very important to achieve an acceptable serum and tissue levels of many nutrients including folate and cobalamin. As we have suggested in a recent paper, even some patients with a high *H pylori* load in their gastric mucosa may show normal serum levels of these vitamins probably because of consuming foods and drinks containing a high level of these vitamins[8].

The main etiologic factors thought to underlie the high prevalence of vitamin B_{12} deficiency in the elderly population are dietary deficiency and malabsorption due to atrophic gastritis. A recent study conducted at our center has confirmed that older age is an independent factor in vitamin B_{12} deficiency, but disproved the malabsorption-atrophic gastritis link since only patients without gastric mucosal atrophy were investigated[32]. The findings of our present study support the positive correlation between age and serum homocysteine level that has been reported previously[33,34]. We suggest that this connection may be explained by a complex interaction among serum vitamin B_{12}, serum folate and erythrocyte folate levels.

In conclusion, even in dyspeptic *H pylori*-positive patients who do not exhibit gastric mucosal atrophy, complete eradication of *H pylori* is associated with a significant drop in serum homocysteine. In countries where *H pylori* infection is highly prevalent, it may be beneficial to implement widespread dietary fortification with folic acid and vitamin

B_{12}, and/or to provide eradication treatment for all infected patients. Further research is needed to determine whether these approaches offer significant clinical benefits in terms of lower cardiovascular risk.

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