Folate and Homocysteine Levels and Their Association with Dietary Intakes in Iranian Patients Infected with *Helicobacter pylori*: a Case-Control Study

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Abstract- The association between *Helicobacter pylori* (HP) infection and concentration of folate or homocysteine are still unclear. The aim of the present study was to assess the effect of HP infection on folate and homocysteine concentrations in patients infected with HP and healthy participants. We also assessed dietary intakes of folate, vitamins B6 and B12 in two groups. In this case-control study, 44 participants with HP-infection and 46 healthy controls were studied. Participants were recruited from those referred to the central laboratory of Tabriz University of Medical Sciences. Blood samples were collected to determine serum folate and homocysteine levels. The presence of both IgG and IgA in serum was considered as HP positive. Dietary intakes were assessed in all participants by 24-hour dietary recalls by trained interviewers for three days. The mean concentration of serum folate was significantly lower in HP-positive patients than in controls (8.49 nmol/L vs. 10.95 nmol/L, respectively; *P*=0.01). Although the mean concentration of serum homocysteine differed between groups, statistical significance was missed (HP infected patients: 9.35 µmol/L; healthy participants: 8.96 µmol/L; *P*=0.064). Macro- and micronutrient intakes showed no significant difference between participants with and without HP infection. In logistic regression models, there was a negative correlation between folate concentration and HP infection even after controlling for confounding factors (OR=0.82; CI95%=0.79-0.97). In this study, authors showed that a negative association presents between HP infection and serum folate concentrations, but the homocysteine status was not differed significantly between HP-positive and HP-negative participants.

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Keywords: Folate; Homocysteine; *Helicobacter pylori*; Dietary intakes

Introduction

Helicobacter pylori (HP) is a microaerophilic human pathogen that is recognized as the major cause of several gastrointestinal diseases such as peptic ulcer, atrophic gastritis and gastric carcinoma (1,2). In developing countries, it is estimated that 70-90% of apparently healthy people are infected with HP (2-4). The infection might disturb the gastric secretions including pepsinogen, gastric acid, intrinsic factor via which it impairs the bioavailability of some B vitamins like vitamin B12 and folate (5,6). Several studies revealed that inadequate intake and low serum concentration of these vitamins can lead to hyperhomocysteinemia (7-9). Increased concentration of plasma total homocysteine have been shown to be a risk factor for cardiovascular disease (CVD) (9) mainly coronary heart disease and stroke (8). Furthermore, in several studies, hyperhomocysteinemia have been associated with cognitive impairment (10) and colorectal cancer (7).

However, the association between folate, homocysteine, and HP infection is still controversial. Although in some studies HP infected patients are

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shown to have lower serum concentration of folate as well as higher homocysteine concentration (5,11,12), others found no associations (1,13,14). In another study, *Helicobacter pylori* eradication reduced homocysteine levels but had no effect on serum folate levels (15). Moreover, serum folate levels were reported as one of the influencing factors of plasma homocysteine levels (11,15).

Taken together, in the present study, authors investigated the association between serum levels of folate, homocysteine and HP infection in patients with HP infection and compared them with healthy participants. The authors also assessed dietary intake of folate, vitamins B_{12} and B_6 in the present study in two groups.

Materials and Methods

This case-control study was conducted in the summer, 2012. The study participants were recruited from the patients who referred to the central laboratory of Tabriz University of Medical Sciences because of gastrointestinal problems. Informed written consent was obtained from all participants. Considering earlier studies about standardization of Ig tests for diagnosis of HP infection (16), authors used the presence of both IgG and IgA in serum of patients as a diagnostic criteria. Exclusion criteria were as follows: 1) (age<25 or >55 years), 2) Patients diagnosed with cardiovascular disease, diabetes mellitus, liver disease, renal failure, celiac disease, inflammatory bowel disease, malabsorption syndrome 3) use of vitamin B supplementation or drugs affecting folate or vitamin B12 homeostasis for six months before the study, 4) use of non-steroidal anti-inflammatory drugs, antibiotics, H2-receptor antagonists, proton pump inhibitors and antacids in the previous month. A total of 44 patients with positive results of both Ig tests were considered as case group. The control group (46 apparently healthy people) was enrolled from patients with negative results in both IgG and IgA tests.

The scientific and ethical issues of this study were approved by Nutrition Research Center of Tabriz University of Medical Sciences (code: 5/71/1670). HP infection was determined by ELISA test (IB L; International GMBH, Germany, Hamburg). Venipuncture collected blood samples. Sera obtained after samples completely clotted by centrifugation at 2000 × g for 10 min. After liquidating sera, the tubes were stored at -80°c until the day of measuring serum folate and homocysteine levels. Serum folate concentration (nmol/L) was assayed by electron chemiluminescence immune assay Elecys system (Roche Diagnostics GmbH, Germany). Homocysteine concentration (µmol/l) was measured by immulite 1000 method (DPC, Los Angeles, CA,USA (1,17).

Weight was measured with light clothes and without shoes to the nearest 0.1 kg using a TANITA digital scale. Height was measured with SECA stadiometer to the nearest 0.1 cm. BMI was calculated using the equation "BMI = weight (kg)/height $(m)^2$". Dietary intakes were assessed using 24-hour dietary recalls by trained interviewers for three days (two workdays and one weekend).

Statistical analysis

Normality of the distribution of data was evaluated by kolmogorov-smirnov test. Data were expressed as mean ± SD for numeric variables. Comparison between groups was evaluated by student's t-test for quantitative and *Chi*-square test or Fisher`s Exact Test for qualitative variables. To evaluate correlations between HP infection and biochemical markers, Spearman (rs) correlation coefficient was used. To predict the association between HP infection and serum folate and homocysteine levels, logistic regression was used by controlling for sex, age, BMI, energy, intakes of vitamins B_6, B_{12}, and folate. All statistical analyzes were performed using Statistical Package for Social Sciences (SPSS) software (version 11.5; SPSS Inc, Chicago, IL, USA). In this study $P,< 0.05$ was considered significant.

Results

The general characteristics of study subjects are shown in table 1. Twenty-two percent of the study subjects were male (11.4% in HP-positive and 32.6% in HP-negative groups).

There were no significant differences in age, BMI and educational status of study groups. Energy, macro- and micronutrient intakes showed no significant differences between participants with and without HP infection (Tables 2 and 3).

Folate and homocysteine Status

Serum folate concentration was higher in HP-negative participants compared with HP-positive ones (10.95 nmol/L vs. 8.49 nmol/L, respectively; P=0.01). However, the mean concentrations of homocysteine were not significantly different between HP infected and healthy participants (9.35 µmol/L vs. 8.96 µmol/L, respectively; P=0.064) (Figure 1).

Association between folate and homocysteine with HP infection

In the logistic regression models with HP infection as a dependent variable, there was an indirect correlation between folate concentration and HP infection (OR=0.87; 95%CI: 0.79-0.97). This correlation was remained significant after controlling for confounding variables (age, sex, BMI, energy, intakes of vitamins B_6, B_{12}, and folate) (OR=0.82; CI 95%: 0.71-0.94). However, no significant association was found between homocysteine status and HP infection even after controlling for confounders (OR= 1.07; 95%CI: 0.93-1.22) (Table 4).

Table 1. Descriptions of the study population with and without HP infection

Variables		HP negative (n=46)	HP positive (N=44)	*P**
BMI (kg /m²)		26.96 ±4.93	25.22±4.06	0.08
Age (year)		38.11±10.5	39.09±10.43	0.65
Sex (%)	Male	15(32.6)	5(11.3)	0.01
	Female	31 (67.4)	39 (88.7)	
Education (%)	None	9(20.5)	6(15.5)	0.51
	Less than high school	19(43.2)	15(38.5)	
	High school	12(27.3)	10(25.5)	
	Post graduate	4(9.0)	8(20.5)	

*: Values for differences between treatment groups derived through Pearson's Chi-Square for categorical variables and Independent t-test for continuous data. BMI: Body mass index

Table 2. Energy and dietary intake of macronutrients in participants with and without HP infection

Variables	Control group (n=46)	Case group (n=44)	*P*.value*
Energy (Kcal/day)	1733.83±506.54	1555.08±570.78	0.13
Protein (g/day)	53.26±16.83	50.51±21.12	0.51
Pro (%)	12.18±1.76	12.80±1.99	0.14
Fat (g/day)	200.87±118.49	201.02±164.20	0.99
Fat (%)	31.49±5.76	30.55±5.57	0.45
SFA (gr)	13.76±5.67	12.34±6.43	0.29
PUFA (gr)	15.54±4.82	15.29±7.11	0.85
MUFA (g/day)	21.67±6.45	19.23±7.55	0.12
Carbohydrate (g/day)	224.10±90.55	222.31±86.75	0.26
Carbohydrate (%)	56.21±5.53	56.36±4.80	0.89
Soluble fiber (g/day)	0.32±0.22	0.30±0.24	0.82
Crude fiber (g/day)	2.51±1.12	2.56±1.05	0.84

*Independent T-Test

Table 3. Dietary intake of micronutrients in participants with and without HP infection

Variables	Control group (n=46)	Case group (n=44)	*P*.value
Vit B_1(mg)	1.60±0.68	1.47±1.06	0.51
Vit$_6$ (mg)	0.661±0.24	0.36±0.28	0.67
Vit C (mg)	72.65±48.37	58.79±39.73	0.15
Ca (mg)	376.21±141.92	323.48±119.62	0.07
Iron (mg)	10.90±3.75	10.50±5.55	0.70
Zinc (mg)	5.37±2.68	4.61±2.09	0.15
Folate (µg)	113.74±51.56	123.01±55.24	0.43
Vit B_{12}(µg)	1.60±0.68	1.47±1.06	0.51

*Independent T-Test

Table 4. Logistic regression model for HP infection as a dependent variable

Variables	B	Odds Ratio	95% CI
Folate	-0.13	0.87	0.79-0.97
Model 1 (age, sex, BMI)	-0.18	0.84	0.74-0.94
Model 2 (model 1 + energy, dietary intake of vitamin B_6, B_{12}, folate)	-0.19	0.82	0.71-0.94
Homocysteine	0.03	1.03	0.92-1.14
Model 1 (age, sex, BMI)	0.42	1.04	0.92-1.17
Model 2 (model 1+ energy, dietary intake of vitamin B_6, B_{12}, folate)	0.07	1.07	0.93-1.22

BMI: Body mass index

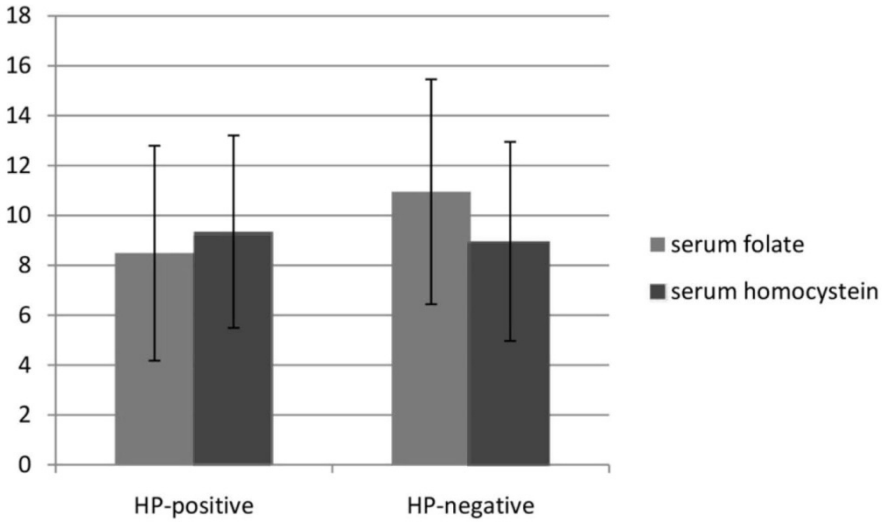

Figure 1. Mean (SD) of folate and homocysteine concentrations (nmol/L) in HP-positive and HP-negative subjects

Discussion

Present study showed that folate concentration in HP-positive participants was significantly lower than HP-negative ones. In current logistic regression models, there was also significant indirect association between folate and HP-infection even after controlling for confounders. This result was in line with other studies (5,18). In a study of patients who underwent diagnostic coronary arteriography, lower levels of folate were determined in HP infected patients. These patients also had more grade of atrophy of stomach (5). However, in a cross-sectional study of Stettin *et al.,* folate levels were nearly the same in HP-infected patients and healthy participants (1). The same results were reported in some other studies (19-24).

Homocysteine is an intermediate product in the metabolism of methionine that its breakdown depends on the presence of vitamin B_{12}, folate and vitamin B6 (25-27). With this regard, some studies indicated that hyperhomocysteinemia might present in HP infection because of the decrement of folate, vitamin B_{12} and B_6 absorption from the diet (1). Hyperhomocysteinemia may cause endothelial dysfunction and morphologic changes in the vascular system (25-27). Hence HP infection indirectly might affect atherosclerosis. However, in this study there was no difference in homocysteine concentration between HP-positive and HP-negative participants. In logistic models, after controlling for confounders, no association was found between HP-infection and hyperhomocysteinemia. In accordance with current results, Itou *et al.,* could not found any association between homocysteine levels and HP infection in 174 patients who met the HP eradication clinic in Nagoya University (14). Hyperhomocysteinemia were not observed in HP-infected volunteers in other studies (1,19-21, 23, 24, 28). In contrast, although not strong, higher homocysteine levels were reported in HP-infected participants in other studies (13,29,30). About this, volunteers in both groups of our study had optimal homocysteine concentrations. Besides, a negative balance of folate was indicated in serum levels less than 7 nmol/L. In some other studies an approximate cut-off value for folate levels in which homocysteine status might be affected reported as 10 nmol/L (31). In present study, participants in both groups had more than 7nmol/ml with HP-negative subjects of 10.95 nmol/L and HP-positive 8.49 nmol/L. In these values, it seems serum levels of folate might have less influence on homocysteine status, explaining

non-difference observed among both groups. In a study in which homocysteine status were affected by serum folate levels, the reported levels of folate were less than7 nmol/L. Furthermore, homocysteine levels were considered to be influenced by atrophic gastritis (20,32,33), in contrast, gastric atrophy may not increase the risk of lower folate levels (14). However, Some authors believe that eradication of HP infection is useful even in the absence of mucosal atrophy (15) especially in countries with lower intake of folate or vitamin B12. Furthermore, Itou *et al.,* did not indicate any association between HP infection and hyperhomocysteinemia in a study of genotypes of folate metabolizing enzymes (14). In this study, *MTHFR* C677T polymorphism was shown to be related to homocysteine status and hyperhomocysteinemia was observed in lower folate level only in participants with TT genotype. The author suggested that in assessing the association between HP infection and homocysteine levels, this genotype should be included.

In the present study, there were no significant differences in folate, vitamin B_{12} or vitamin B_6 intakes between HP-positive and negative participants. To our knowledge, there was not any study to assess dietary intake of nutrient intakes in HP- infected patients if any. Earlier study showed supplementation of folate enriched diet (200 μg/day) resulted in the reduction of homocysteine levels in comparison with the control group (8). In current study, the folate intake of all participants were less than RDA (<400 μg/day). So, there is a need to increase dietary intake of this micronutrient by achieving more amount of folate-rich foods like green leafy vegetables.

The present study had some limitations. Upper esophagogastroduodenal endoscopy is considered the reference method of diagnosis of HP infection. In present study, we used two ELISA test for detection of IgG and IgA for the HP diagnosis. The standardized ELISA tests for IgG and IgA was shown to have 93.2 and 95 sensitivity and specificity, respectively (16). This method is preferred because it was non-invasive, economical and rapid. Moreover, in this study, we could not match the case and control group according to sex. However, in regression models, using sex as a confounder did not change the results. Besides, we did not essay the atrophy of the stomach in our study participants. Some studies showed that hyperhomocysteinemia might present only in atrophic gastritis (5, 8). It is suggested to measure the grade of atrophy in the future studies. Present study had some strength too. We used logistic regression to control confounders in our models. Besides, we assessed micro and macronutrient intakes in our groups to control the effect of diet on vitamin status.

In conclusion, the present study showed that there was a negative association between HP infection and serum folate levels, but the homocysteine status were not differed significantly in both HP-positive and HP-negative participants. Also, there was no significant difference in dietary intakes of folate, vitamins B12 and B6 between the study groups.

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References

1. Stettin D, Waldmann A, Strohle A, et al. Association between Helicobacter pylori infection, C-reactive protein and status of B vitamins. Adv Med Sci 2008;53(2):205-13.
2. Bahrami AR, Rahimi E, Ghasemian Safaei H. Detection of Helicobacter pylori in City Water, Dental Units' Water, and Bottled Mineral Water in Isfahan, Iran. Scientific World J 2013;2013:280510.
3. Cave DR. Transmission and epidemiology of Helicobacter pylori. Am J Med 1996;100(5A):12S-7S.
4. Rautelin H, Kosunen TU. Helicobacter pylori and associated gastroduodenal diseases. APMIS 1991;99(1):677-95.
5. Tamura A, Fujioka T, Nasu M. Relation of Helicobacter pylori infection on plasma vitamin B, folic acid, and homocysteine levels in patients who underwent diagnostic coronary arteriography. Am J Gastroenterol 2002;97(4):861-6.
6. Redeen K, Stefan R, Anna P, et al. Homocysteine levels in chronic gastritis and other conditions: relations to incident cardiovascular disease and dementia. Dig Dis Sci 2010;55(2):351-8.
7. Kim YI. Folate and colorectal cancer. An evidence□based critical review. Mol Nutr Food Res 2007;51(3):267-92.
8. Zappacosta B, Mastroiacovo P, Persichilli S, et al. Homocysteine Lowering by Folate-Rich Diet or Pharmacological Supplementations in Subjects with Moderate Hyperhomocysteinemia. Nutrients 2013;5(5):1531-3.
9. Wald DS, Law M, Morris JK. Homocysteine and

cardiovascular disease: evidence on causality from a meta-analysis. Bmj 2002;325(7374):1202.
10. Gopinath B, Flood VM, Rochtchina E, et al. Homocysteine, folate, vitamin B-12, and 10-y incidence of age-related macular degeneration. Am J Clin Nutr 2013;98(1):129-35.
11. Hamatani R, Otsu M, Chikamoto H, et al. Plasma homocysteine and folate levels and dietary folate intake in adolescents and young adults who underwent kidney transplantation during childhood. Clin Eep Nephrol 2014:18(1):151-6.
12. Lewerin C, Jacobsson S, Lindstedt G, et al. Serum biomarkers for atrophic gastritis and antibodies against Helicobacter pylori in the elderly. Implications for vitamin B12, folic acid and iron status and response to oral vitamin therapy. Scand J Gastroenterol 2008;43(9):1050-6.
13. Rasool S, Abid S, Igbal MP, Mehboobali N, Haider G, Jafri W. Relationship between vitamin B12, folate and homocysteine levels and H. Pylori infection in patients with functional dyspepsia: A cross-section study. BMC Res Notes 2012;5(1):206.
14. Itou S, Goto Y, Kondo T, Nishio K, Kawai S, Ishida Y, Naito M, Hamajima N. No associations of Helicobacter pylori infection and gastric atrophy with plasma total homocysteine in Japanese. Int J Med Sci 2007;4(2):98-104.
15. Ozer B, Serin E, Gumurdulu Y, et al. Helicobacter pylori eradication lowers serum homocysteine level in patients without gastric atrophy. World J Gastroenterol 2005;11(18):2764-7.
16. Sarafnezad A, Hoodei E, Siavoshi F, Maserrat S, Jadali Z, Shahrestani T. Standardization and in-house ELISA setup for helicobacter pylori serologic diagnosis. Tehran Univ Med J 2001;59(4):1-10.
17. Redeen S, Ryberg A, Petersson F, Eriksson O, Nagge K, Borch K. Homocysteine Levels in Chronic Gastritis and Other Conditions. Relations to Incident Cardiovascular Disease and Dementia. Dig Dis Sci 2010;55(2):351-8.
18. Markle HV. Coronary artery disease associated with Helicobacter pylori infection is at least partially due to inadequate folate status. Med hypotheses 1997;49(4):289-92.
19. Triantafillidis J, Georgakopoulos D, Gikas A, et al. Relation between Helicobacter pylori infection, thyroid hormone levels and cardiovascular risk factors on blood donors. Hepato-Gastroenterology 2003;50(Suppl 2):cccxviii-cccxx.
20. Santarelli L, Gabrielli M, Cremonini F, et al. Atrophic gastritis as a cause of hyperhomocysteinaemia. Aliment Pharmacol Ther 2004;19(1):107-11.
21. Trimarchi H, Forrester M, Schropp J, et al. Low initial vitamin B12 levels in Helicobacter pylori-positive patients on chronic hemodialysis. Nephron Clin Pract 2004;96(1):c28-c32.
22. Shuval-Sudai O, Granot E. An association between Helicobacter pylori infection and serum vitamin B12 levels in healthy adults. J clin gastroenterol 2003;36(2):130-3.
23. Bloemenkamp D, Mali W, Tanis B, et al. The relation between Helicobacter pylori and atherosclerosis cannot be explained by a high homocysteine concentration. Eur J Clin Invest 2002;32(8):549-55.
24. Saxena V, Markus H, Swaminathan S, et al. Hyperhomocysteinaemia, Helicobacter pylori, and coronary heart disease. Heart 1997;78(5):524.
25. Wolters M, Strohle A, Hahn A. Cobalamin: a critical vitamin in the elderly. Prev Med 2004;39(6):1256-66.
26. Wolters M, Strohle A, Hahn A. Age-associated changes in the metabolism of vitamin B (12) and folic acid: prevalence, aetiopathogenesis and pathophysiological consequences. Z Gerontol Geriatr 2004;37(2):109-35.
27. Stanger O, Herrmann W, Pietrzik K, et al. DACH-LIGA homocystein (german, austrian and swiss homocysteine society): consensus paper on the rational clinical use of homocysteine, folic acid and B-vitamins in cardiovascular and thrombotic diseases: guidelines and recommendations. Clin Chem Lab Med 2003;41(11):1392-403.
28. Sung JJ, Sanderson JE. Hyperhomocysteinaemia, Helicobacter pylori, and coronary heart disease. Heart 1996;76(4):305-7.
29. Cenerelli S, Bonazzi P, Galeazzi R, Testa I, Bonfigli A, Sirolla C, Giunta S, Galeazzi L, Fumelli D, Testa R. Helicobacter pylori masks differences in homocysteine plasma levels between controls and type 2 diabetic patients. EurJ Clin Invest 2002;32(3):158-62.
30. Whincup P, Danesh J, Walker M, Lennon L, Thomson A, Appleby P, Hawkey C, Atherton J. Prospective study of potentially virulent strains of Helicobacter pylori and coronary heart disease in middle-aged men. Circulation 2000;101(14):1647-52.
31. Quinlivan E, Partlin J, Nulty H, et al. Importance of both folic acid and vitamin B12 in reduction of risk of vascular disease. Lancet 2002;359(9302):227-8.
32. Yamada T, Searle JG, Ahnen D, et al. Helicobacter pylori in peptic ulcer disease. JAMA 1994;272(1):65-69.
33. Sipponen P, Laxen F, Huotari K, et al. Prevalence of low vitamin B12 and high homocysteine in serum in an elderly male population. Association with atrophic gastritis and Helicobacter pylori infection. Scan Gastroenterol 2003;38(12):1209-16.