Effect of Folic acid on Serum Homocysteine and Morbidity in Patients with Chronic Coronary Artery Disease

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Abstract

Background- In addition to traditional cardiovascular risk factors, high levels of plasma homocysteine has been documented recently as independent risk factors for atherosclerosis. The probable mechanism is through endothelial dysfunction. Roughly 10% of the population with coronary artery disease (CAD) may have hyper-homocysteinemia. Since folic acid is a potential factor in lowering plasma homocysteine and dietary intake of folic acid is not sufficient, it needs to be prescribed to CAD patients as a supplement. The purpose of this study is to assess the effect of folic acid on plasma homocysteine levels and on morbidity in stable CAD patients.

Methods- In this prospective interventional study, we recruited 52 stable CAD patients; the plasma levels of homocysteine, folic acid and vitamin B12 were measured. The morbidity-related indices (the number of sublingual TNGs per week, typical anginal chest pain per week, the number of cardiovascular-related hospitalizations in the previous 3 months, functional class and ECG changes) were determined. All patients received 2 mg oral folic acid daily for 3 months. At the end of the study, the level of homocysteine and morbidity were determined.

Results- Folic acid supplementation for 3 months was associated with a decrease in homocysteine level by 44% (P=0.000). We did not observe a significant change in levels of serum folic acid. There were significant declines in all morbidity indices including TNG consumption, frequency of chest pain, functional class and hospitalizations (P=0.001).

Conclusion- The findings indicate that 2 mg folic acid orally daily for 3 months is associated with a decrease in homocysteine level and morbidity in CAD patients ***(Iranian Heart Journal 2007; 8 (2): 44-50).***

Key words: serum homocysteine ■ folic acid ■ morbidity ■ coronary artery disease

Coronary artery disease (CAD) is a major cause of disability and mortality in developed and developing countries. In addition to classic risk factors for CAD, increased homocysteine level as an independent risk factor for atherosclerosis has been documented in several studies recently.[1]

The mechanism of occurrence of CAD with increasing serum hemocysteine is unknown. The probable mechanism is through endothelial dysfunction and predisposing to thrombosis. In this regard, the excessive oxidation of LDL cholesterol, dysfunction in vasodilator factors derived from endothelium and decreased dilating power of arteries should not be ignored.[2]

The prevalence of hyper-homocysteinemia in the general population was 5 to 10%, even 30

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to 40% in old age groups and it increases the risk of CAD up to 10% as an independent risk factor. Increased homocysteine was primarily due to decreased intake of folic acid.[2] Screening tests have been suggested for diagnosis of increased serum homocysteine (≥15µmol/L) in advanced atherosclerosis despite normal lipoprotein levels and in absence of other risk factors in young patients with a family history of atherosclerosis.[3] It has been shown that high level of homocysteine is related with high prevalence in CAD patients with at least one risk factor.[4]

In general, the cut-off point of desirable homocysteine level is <12µmol/L and homocysteine level of 12-15µmol/L is the boundary limit and >15µmol/lit is associated with the risk of CAD. The latter level increases the risk of atherosclerosis by 1.5 to 2 times. However the increasing level of homocysteine can be treated effectively with supplements of folic acid in the nutrition.[5] Using folic acid and anti-oxidants (vitamin E, C, A, B12) can decrease serum homocysteine and thus lead to lowering oxidation of LDL in CAD patients.[6] In addition to independent effects of folic acid on homocysteine levels, it reverses the endothelial dysfunction of arteries in CAD patients[7-10] and thus it can decrease the mortality of ischemic heart disease.[12]

In published studies, the effective dose of folic acid recommended was between 400 and 500µg/day; with this dose the decrease in homosysteine level was about 25-30%,[2,11] and adding vitamin B12 to this regimen gave an additional decrease in homosysteine. Although the recommended standard dose is 200µg/day, in many studies the higher dose was often used in order to achieve the desirable rate of decrease in homosysteine level.[2,12-15] Even in patients who do not suffer from lack of vitamins, folic acid leads to a decline in serum homosysteine level.[17] Since increased use of acid folic may induce symptoms of vitamin B12 deficiency, thus all supplementation regimens including folic acid of 400µg/day suggest adding this vitamin to the therapeutic regimen.[2] However, the recommended dose is still controversial. This interventional study was aimed at assessing the effect of folic acid 2mg/day for 3 months on homocysteine levels in CAD patients.

Methods

We conducted a prospective interventional study on 67 patients with stable coronary artery disease. First, we recruited 67 patients with a diagnosis of stable CAD. Our exclusion criteria were: 1), the patient who had recent myocardial infarction or unstable angina, 2), patients who used the vitamin supplementation before recruitment, 3) patients treated with anti-folate drugs, e.g. chemotherapy, 4) patients with renal disorder (creatinine >1.5 mg/dl), 5) patients who do not written consent for study regimen, 6) patients with lack of time in using the recommended regimen and 7) patients with intolerance in using folic acid.

Out of 67 recruited subjects under study, 15 patients were excluded because of lack of using our therapeutic regimen and failing to return for following laboratory tests. Thus the data of 52 subjects were analyzed, of whom 36 had history of old MI or unstable angina, and 36 subjects with stable chronic angina. In all subjects, coronary artery disease was confirmed with clinical examination, history of ECG changes, exercise test, thallium scan and angiography in some cases. In all recruited subjects, first the investigator explained the reason for the study, and the benefit and the risk were mentioned and all subjects gave a written consent.

First, before starting the intervention program, the level of serum homocysteine, folic acid and vitamin B12 were measured in the same laboratory unit in all subjects. For determining the morbidity, we used the indices of number of nitroglycerines used per week, number of chest pain attacks of typical angina per week, number of hospitalizations because of heart problems during 3 months, functional class and ECG. The serum

homocysteine was measure with ELISA method (Axis, IBL, Gemany) and also the level of serum folic acid and vitamin B12 were determined with radioimmunoassay (TCN, USA). The normal range of homocysteine was 5-15µmol/lit and also the normal limits of folic acid and vitamin B12 were 1.5-16.9ng/ml and 160-970pg/ml, respectively. Then, all patients were given oral folic acid tablets 2 mg/day for 3 months. After 3 months, the serum homocysteine, folic acid and vitamin B12 were measured again in the same laboratory unit and also the morbidity indices were determined. The data were analyzed using SPSS software. In statistical analysis, we used paired t-test and matched pair Wilcoxon test. The P-value < 0.05 was considered as statistically significant.

Results

The results in Table I show that the mean± SD of fasting homocysteine (Hcy) was 12.37± 10.3µmol/L before treatment with folic acid. This is higher than the upper limit of normal. After treatment with folic acid, the mean Hcy significantly decreased to 6.92±3.06µmol/L (P<0.001) and the decrease was 44%. The mean folic acid tended to decrease slightly about 0.6ng/ml, but it was not statistically significant (P=0.19). Also we did not observe a significant difference in the mean vitamin B12 level before and after treatment. In subgroup analysis, table II shows that the effect of folic acid on homocysteine level was greater when the baseline Hcy≥15µmol/L compared with Hcy<15 µmol/L and the difference effect was 14.72 (P<0.001) and 0.95 (P<0.01) respectively in the two groups. In addition, we observed that the effect of folic acid on homocysteine level was higher in men versus women (the mean differences were 6.63, P<0.001 and 3.31µmol/L, P=0.04), respectively (Table III). Table IV shows that all four morbidity indices were significantly decreased after 3 months of consumption of folic acid and the effect of folic acid was greater on the number of chest pain attacks and number of TNGs used per week as compared to the two other morbidity indices (number of hospitalizations and the functional class).

Table I. The number of patients and the mean (± SD) of homocysteine, folic acid and vitamin B_{12} before and after treatment.

Variables	No.	Before treatment Mean ± SD	After treatment Mean ± SD	Mean Difference	P value
Homocysteine µm/l	52	12.37 ±10.03	6.92±3.06	5.45	0.000
Folic acid ng/ml	52	10.36± 3.93	9.76±3.38	0.6	0.19
Vitamin B_{12}	52	426.27 ± 128.37	420.38±122.62	5.88	0.75

Table II. The number of patients and the mean (± SD) of homocysteine, folic acid and vitamin B_{12} before and after treatment with respect to baseline homocysteine level.

Variables	Hcy status	No.	Before treatment Mean ± SD	After treatment Mean ± SD	Mean Difference	P value
Homocysteine (µm/l)	Hcy≥ 15	17	24.37±8.58	9.64± 3.23	14.72	0.000
Folic acid (ng/ml)		17	10.91±3.57	9.15±3.30	1.76	0.079
Vitamin B_{12}		17	417.65±133.31	400.65±125.07	17.0	0.632
Homocysteine (µm/l)	Hcy<15	35	6.54±3.07	5.60 ±1.90	0.95	0.008
Folic acid (ng/ml)		35	10.09±3.95	9.34±3.34	1.07	0.065
Vitamin B_{12}		35	430.82±119.16	405.91±109.13	24.91	0.20

Table III. The number of patients and the mean (± SD) of homocysteine, folic acid and vitamin B_{12} before and after treatment with respect to sex.

Variables	Sex	No.	Before treatment Mean±SD	After treatment Mean±SD	Mean Difference	P value
Homocysteine (µm/l)	Male	34	13.92±10.63	7.28±3.15	6.63	0.000
Folic acid (mg/ml)		34	10.41±3.95	4.34±3.34	1.07	0.065
Vitamin B12		34	430.82±119.16	405.41±109.13	24.9	0.20
Homocysteine (µm/l)	Female	18	9.45±8.28	6.23±2.82	3.21	0.043
Folic acid(mg/ml)		18	10.26±3.98	10.55±3.41	-0.29	0.69
Vitamin B12		18	417.67±147.49	447.72±144.16	-30.06	0.45

Table IV. The mean of morbidity indices before and after treatment.

Morbidity indices	Before treatment Mean±SD	After treatment Mean±SD	Mean Difference	P value
No of TNG/ week	1.08±1.56	0.6±0.92	0.48	0.000
No. of chest pain attack	1.4±1.9	0.6±0.93	0.81	0.0001
No of hospitalizations	0.33±0.81	0.17±0.47	0.51	0.01
Functional class	1.73±0.67	1.51±0.58	0.22	0.001

Discussion

The findings of this study showed that folic acid induced a decrease of homocysteine (Hcy) levels of up to 44%, and also it caused a decrease in the morbidity of CAD patients. In published studies, the occurrence of atherosclerosis corresponded with Hcy>14µmol/L[19] and thus Hcy≥10µmol/L was considered the upper normal limit. In our study, the mean (±SD) of fasting Hcy before treatment with folic acid was 12.27 ± 10.03µmol/L, that it higher than the upper normal limit and after supplementation of folic acid 2mg/day, we observed a significant decrease in Hcy level to the normal range of up to 6.29µmol/L and the average decrease was 5.45µmol/L. Based on published studies, there is no concordance on the optimal dose of folic acid for decreasing on serum Hcy but different studies used a range of doses of 0.5mg/day to 10mg/day.[19] Our study dose was 2mg/day, that is an intermediate dose was used.

In comparison with other studies, our results are consistent with those reported by Boushey et al. (1995) in a metaanalysis of 12 studies that revealed daily intake of folic acid as a supplement reduced serum Hcy significantly.[18] Thambyrajah (2001) in a randomized controlled clinical trial showed that daily consumption of folic acid corresponded with a significant decrease in Hcy level in CAD patients.[10] Kum et al. (2002) on 29 subjects showed high doses of folic acid in long-term consumption decreased homocysteine levels significantly. In comparison to our study, we used much lower doses than they used (2mg/day vs. 10mg/day). Luszlo et al (2002) on 20 subjects with CAD reported that 9mg/day folic acid for 3 weeks decreased the mean serum Hcy by 4.87µmol/L (26.5%). As in our study, the baseline Hcy in their study was 15.6 (6.1) µmol/L.[20] However, the decreasing rate of Hcy in our study (44%) was greater than those reported in published data in different studies. This difference may be explained by variation in geography, race and usual consumption of folic acid.

In another controlled randomized trial, a 23% reduction in serum homocysteine level was found by prescribing folic acid 0.8mg/day for 4 months.[12] Although the prescribed dose of

our study is higher than this RCT, the author of this trial reported that doses higher than 0.8mg/day have similar effects in reduction of homocysteine level.[12] In a meta-analysis, Clark et al. (2003) reported that the effect of folic acid with doses of 0.5 to 5 mg/day was similar and the reduction rate of homocysteine level was up to 25% and their baseline homocysteine was the same as ours; 12µmol/L.[21] Van Oort et al. (2003) however found the least dose of 400microg/day decreased homocysteine level up to 22% and the effect of higher doses was greater than this least effect. Lein et al., in a RCT study of folic acid with 300 patients with stable CAD for 24 months, found the reduction of homocysteine level was 18% with folic acid and there was no reduction of homocysteine level in the control group[23] and the baseline homocysteine level was similar to ours (12µmol/L). In other published studies,[6,8,24,25] similar results in reduction of homocysteine levels were found. Our finding was consistent with published data that almost confirmed the reduction in homocysteine level will occur with folic acid, but the rate of reduction varied in different studies. Whether the reduction of homocysteine level with folic acid leads to the reduction of coronary events is controversial.[2,12,21]

In subgroup analysis, our findings revealed that the effect of reduction in homocysteine level by folic acid was greater when the baseline homocysteine level was higher than the normal range (≥15 µmol/lit) as compared to those with baseline levels of homocysteine within normal limits. The different published studies also support these findings.[12,13,19,21,26,27]

The findings of our study also showed that the effect of folic acid in decreasing serum homocysteine level was greater in men than women. This might be explained by the higher baseline plasma homocysteine level of men compared to women[2] which was also revealed in our study. In fact, the effect of folic acid is related to the baseline serum homocysteine level. The spectrum of response rate of homocysteine in relation to folic acid is varied in different subjects and in one report, 47.6% of its variation is explained by baseline homocysteine level, gender, smoking status and polymorphism.[28]

In terms of the effect of folic acid on morbidity indices, we found its effect on the number of chest pain attacks and number of TNGs used sublingually per week was greater than other indices such as number of hospitalizations for 3 months, variation of functional class and ECG. Walds et al. (2001) reported that folic acid, through the mechanism of decreasing homocysteine level, yields a decline in morbidity of ischemic heart disease.[12] In contrast, Liema (2001) reported that in spite of decreased homocysteine level by folic acid, the prognosis of stable CAD patients did not change.[12]

At the end of our study, the folic acid level tended to decrease, but did not achieve statistical significance. In published studies it was reported that the level of folic acid was increased significantly.[6,8,19,20,24] The differences might be the low dose of folic acid used in our study compared with others. The decreasing rate might be due to sampling variation and we definitely do not attribute it to lack of compliance of patients to folic acid because a significant decrease of homocysteine level was related to folic acid in our study. Probably the decrease in folic acid in our study may be related to other unknown mechanisms that need to be clarified in the future.

Our findings indicate that prescribing folic acid 2mg/day for a short time (3 months) decreases significantly serum homocysteine levels and morbidity indices. Thus we recommend prescribing folic acid to all chronic CAD patients without considering their baseline homocysteine level.

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