# Effect of Different Doses of Folic Acid on Serum Homocysteine Level in Patients on Hemodialysis

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**Introduction.** Hyperhomocysteinemia is common in patients with end-stage renal disease. It is one of the risk factors for cardiovascular disease. We evaluated the effect of different doses of folic acid on serum homocysteine level in patients on hemodialysis.

**Materials and Methods.** Patients on maintenance hemodialysis were randomized into 4 groups to receive oral folic acid at doses of 2, 5, 10, and 15 mg/d, each for a period of 4 weeks. Serum homocysteine level was measured in all of the patients before and at the end of each week of therapy. Folic acid supplementation was discontinued during a washout period of 1 week between each of the four phases of the trial.

**Results.** Thirty-one hemodialysis patients completed the four phases of treatment with each dose of folic acid (17 women and 14 men). The mean age of patients was 57.6 ± 14.6 years. Serum homocysteine level was reduced significantly compared to its basal level after treatment with folic acid at different doses ($P < .001$). Different doses of folic acid were not significantly different in lowering serum homocysteine levels.

**Conclusions.** Our study failed to show any difference between high-dose and low-dose folic acid therapy regarding their effect on serum homocysteine level. It seems folic acid, 2 mg/d, is an adequate dose, and there is no need to administer a higher dose of it.

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## INTRODUCTION

The prevalence of end-stage renal disease (ESRD) is increasing in the Unite States. Despite many efforts from a consultation committee to maintenance therapy and improved quality of hemodialysis, ESRD has an outstanding morbidity and mortality. Survival rate of patients after 1, 2, 5, and 10 years being on hemodialysis were 80%, 67%, 40%, and 18%, respectively.[1]

Cardiovascular disease (CVD) is the leading cause of mortality in patients with ESRD, which accounts for 50% of mortality rates. The high level of morbidity and mortality occurs while at the same time, the rate of coronary artery diseases is increasing in the general population. Furthermore, some risk factors such as diabetes mellitus, hypertension, hyperlipidemia, and anemia can exacerbate preexisting conditions, especially coronary artery diseases. Patients in different stages of chronic kidney diseases that are not still dependent on dialysis have a greater risk for CVD.[2]

High serum level of homocysteine in patients with ESRD is one of the risk factors for CVD. Reduced serum level of some necessary cofactors in homocysteine metabolism as well as alterations in the vascular endothelial system may lead to atherosclerosis and thrombosis in patients with ESRD. In a study on dialysis patients with and without cardiovascular diseases, serum homocysteine level was significantly high in patients with CVD compared to patients without accompanying CVD (37.2 µmol/L versus 24

μmol/L). In another study performed on 176 patients with ESRD, patients with a greater serum level of homocysteine had 2.9 times higher rates of atherosclerosis and thrombotic events.[2] One study showed that supraphysiologic levels of cofactors in homocysteine metabolism, including folic acid, 15 mg/d, vitamin B6, 100 mg/d, and vitamin B12, 1 mg/d, could keep serum homocysteine level lower than its normal serum level.[2] However, in the absence of any thrombotic events, there is no definite indication for treatment of hyperhomocysteinemia.[3]

Despite many different studies, an established dose to reduce the serum level of homocysteine has not been determined yet. Considering the important pathophysiologic role of hyperhomocysteinemia in patients with ESRD, we aimed to assess the effects of different doses of folic acid as a cofactor of homocysteine metabolism on its serum level in hemodialysis patients. Finding an appropriate dose of folic acid for better monitoring and lowering the burden of CVD in this population is necessary.

## MATERIALS AND METHODS

Thirty-six patients on maintenance hemodialysis who were referred to Fatemieh hospital in Semnan, Iran, were enrolled in this randomized cross-over trial. The exclusion criteria were age under 20 years; alcohol consumption; smoking; recent use of methotrexate, trimethoprim, phenytoin, carbamazepine, or theophylline; and underlying diseases such as malignancy, systemic diseases, systemic lupus erythematosus, rheumatoid arthritis, and hypothyroidism. All of the patients were on dialysis by polysulfone membranes and the Kt/V was between 1.2 and 1.5. Demographic and clinical data of all participants were recorded, including age, duration of hemodialysis, numbers of weekly hemodialysis sessions, underlying diseases, medication history, smoking, and alcohol consumption.

All of the participants discontinued taking folic acid for a week. They were divided randomly into 4 groups of 9 persons. Randomization was performed by using the table of random numbers. Supplementation with oral folic acid was initiated at doses of 2, 5, 10, and 15 mg/d for the patients in groups 1 to 4, respectively. On the days of dialysis, the patients would take the supplement in the presence of a physician and on the other days, tablets count was used to monitor the adherence to treatment. To minimize the possible interactions of confounding factors, vitamin B12, 1000 U/w, was intravenously administered to all of the participants during the study.

All the four studied groups received the scheduled treatment for 4 weeks. At the end of the 4th week, serum homocysteine level was measured. As serum homocysteine half life is about 18 to 25 hours,[3] folic acid was discontinued for 1 week (washout period). Thereafter, the four groups started a different scheduled dose of folic acid supplementation for another 4 weeks. These 4-week periods continued until all groups received all doses of 2, 5, 10, and 15 mg/d of folic acid supplementation.

To determine serum homocysteine level, after a washout period of 1 week, 3 mL of blood was drawn and centrifuged. Serum level of homocysteine was measured by the FHCY100 Axis Homocysteine enzyme immunoassay kit (Axis-Shield, Dundee, Scotland).

Data analysis was performed using SPSS software (Statistical Package for the Social Sciences, version 11.5, SPSS Inc, Chicago, Ill, USA). The 1-way repeated measure analysis of variance was used for analyses. A *P* value less than .05 was considered significant.

## RESULTS

Of 36 patients enrolled in this study, 2 did not complete the course of the study and 3 died. Therefore, data was collected from 31 hemodialysis patients participated in the entire course of this study. Seventeen patients (54.8%) were women and 14 (45.2%) were men. The mean age of the patients was 57.6 ± 14.6 years (range, 33 to 88 years; Table 1). Regarding the underlying disease, 11 patients (35.5%) were hypertensive, 10 (32.3%)

**Table 1.** Distribution of Age, Sex and Baseline Homocysteine Level in Hemodialysis Patients*

| Characteristic | Men | Women | All |
|---|---|---|---|
| Number of patients | 14 | 17 | 31 |
| Mean age, y | 57.6 ± 16.5 | 56.7 ± 14.2 | 57.6 ± 14.6 |
| Mean serum homocysteine level, μmol/L | 17.0 ± 5.1 | 18.3 ± 8.1 | 18.2 ± 6.4 |

*Values for age and homocysteine are mean ± standard deviation.

**Table 2.** Serum Homocysteine Level After Supplementation With Folic Acid*

| Folic Acid Group | Homocysteine, µmol/L | | P† |
|---|---|---|---|
| | Mean | 95% Confidence Interval | |
| 2 mg | 5.1 ± 0.8 | 2.7 to 7.5 | < .001 |
| 5 mg | 5.5 ± 1.1 | 2.0 to 9.0 | < .001 |
| 10 mg | 4.8 ± 0.7 | 2.6 to 7.1 | < .001 |
| 15 mg | 5.6 ± 1.2 | 2.1 to 9.2 | < .001 |

*Values for age and homocysteine are mean ± standard deviation.
†Homocysteine levels were compared with the baseline levels.

had diabetes mellitus, 2 (6.5%) were known to have Alport syndrome, and 8 (25.7%) did not have any known underlying cause of the ESRD. All of the patients were on hemodialysis 3 times a week. The mean duration of being on hemodialysis was 2.6 ± 2.2 years (range, 0.6 to 9 years), and 35.5% of the patients were on maintenance hemodialysis for more than 2 years. The mean hemoglobin concentration was 11.7 ± 1.6 g/dL (range, 6.5 g/dL to 15.2 g/dL), and 6.5% of the patients had a serum hemoglobin level of less than 10 g/dL. The mean serum homocysteine level of the studied patients before the beginning of the folic acid therapy was 18.2 µmol/L. Serum homocysteine levels returned to values within the reference range (5 µmol/L to 15 µmol/L) in all the four studied groups during the washout periods. After treatment with the doses of 2, 5, 10, and 15 mg/d of folic acid, the mean reduction of serum homocysteine level was 5.1, 5.5, 4.8, and 5.6 µmol/L, respectively. While the patients showed a significant decrease in serum homocysteine level after treatment with any dose of folic acid as compared to the serum baseline level ($P < .001$), serum homocysteine levels did not show a significant difference after treatment with different doses of folic acid (Table 2).

## DISCUSSION

We showed a significant decrease in serum homocysteine level of hemodialysis patients after supplementation with any dose of folic acid from 2 mg/d to 15 mg/d ($P < .001$); however, none of the doses had a privilege over the other ones. The effect of treatment with folic acid on the plasma level of homocysteine in dialysis patients is controversial. In a study by De Vecchi and colleagues, after a 2-month therapy with folic acid, 5 mg/d, the plasma homocysteine level in dialysis patients reduced significantly, but it did not reach the normal level.[4] Other studies also showed a reduction of the homocysteine level with the folic acid, but homocysteine level never normalized.[5] The reason would be corresponded to the baseline homocysteine level of their studied population, which might be higher than our study participants'. Accumulation of the cysteine sulfinic acid is another possibility.[6]

Righetti and coworkers[7] used folic acid at a dose of 15 mg/d. Following treatment, serum homocysteine level decreased to 33.6% of the baseline level, similar to our study findings. Manns and colleagues[8] showed a significant reduction of serum homocysteine level in hemodialysis patients after taking folic acid, 5 mg/d and 20 mg/d; however, same as our findings, they did not find any significant difference in serum homocysteine level after supplementation with different doses of folic acid. In the study by Hyndman and coworkers,[9] substantial reduction of the serum homocysteine level was not reported after administration of a high dose of folic acid. Folic acid supplementation at a high dose, ie, greater than 1, 5, and 20 mg/d did not significantly alter serum homocysteine level. The difference in baseline serum homocysteine levels along with supplementation with vitamin B12 that was prescribed to the hemodialysis patients in our study is among the probable contributing factors to reach different results. A study by Dierkes and associates[6] showed there was no difference between folic acid supplementation at a dose of 5 mg to 25 mg and 1 mg to 5 mg to reduce serum homocysteine level. Supplementation with 2.5 mg and 5 mg of folic acid for 4 weeks did not normalize the serum homocysteine levels in dialysis patients. In addition, they showed that hemodialysis did not have any significant effect on the serum homocysteine level. Tremblay and colleagues[10] assessed the supplementation effects of intravenous folic acid, 10 mg, 3 times per week. They found that folic acid at this dose had the same effect as folic acid at a dose of 1 mg/d. In a study by Bernasconi and coworkers, 5-mg and 15-mg doses of daily folic acid significantly reduced serum homocysteine level in hemodialysis patients, but there was no significant difference between the two different doses, which is similar to our findings.[11]

Arnadottir and colleagues[12] studied supplementation effects of different doses of folic acid, including 5 mg, 3 times per week, for 6 weeks, 5 mg/d for 6 weeks, and 10 mg/d for 6 weeks. They

found that more reduction in serum homocysteine level was achieved by administering 5 mg of folic acid, 3 times per week (15 mg/w). This discrepancy between results of their study and ours might be explained by the duration of supplement therapy. They assessed for supplementation effects for a longer period, which was contrary to our findings (6 weeks versus 4 weeks). In addition, in their study, the level of homocysteine in every stage of treatment never reached normal values. Finally, Sunder-Plassmann and colleagues[13] compared 15-, 30-, and 60-mg/d doses of folic acid, and they found no beneficial effects between different doses in reducing the serum homocysteine level. It seems that saturation of erythrocytes with folate is one of the probable mechanisms that is contributed to reach unsatisfactory effects following treatment with high-dose folic acid.[14]

We did not encounter any side effects due to folic acid supplementation, including allergic reactions, bronchospasm, erythema, rash, flushing, malaise, and pruritus in our study. However, the small sample size is one of the limitations in our study. We controlled patients' adherence by counting the number of tablets and consultation 3 times a week, but it does not seem to be the perfect and fully supported assessment method. We conducted a 4-week duration therapy because of the short half-life of folic acid and normalization of its serum level after the washout period of 1 week. However, study with a large sample size and longer duration and follow-up period is recommended.

## CONCLUSIONS

In this study, we found that supplementation therapy with folic acid significantly reduced the serum homocysteine level. However, there was no significant effect comparing low-dose and high-dose folic acid supplementation. It seems that prescribing folic acid at a dose of 2 mg/d in hemodialysis patients is adequate, and there is no need to administer high-dose folic acid.

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## CONFLICT OF INTEREST

None declared.

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