Comparative Study of Response to Treatment with Supraphysiologic Doses of B-Vitamins in Hyperhomocysteinemic Hemodialysis Patients

Farid Nakhoul MD[1,3], Zaid Abassi PhD[3], Michal Plawner MD[2], Eli Khankin MD[1], Rawi Ramadan MD[1], Noami Lanir PhD[2], Benjamine Brenner MD[2,3] and Jacob Green MD[1,3*]

Departments of [1]Nephrology and [2]Hematology, Rambam Medical Center, Haifa, Israel
[3]Technion Faculty of Medicine, Haifa, Israel

Key words: homocysteine, hemodialysis, folic acid, B-vitamins, methylenetetrahydrofolate reductase, vascular access thrombosis, diabetes

Abstract

Background: Hyperhomocysteinemia is a well-recognized risk factor for accelerated atherosclerosis in hemodialysis patients.

Objectives: To examine the effects of two doses of vitamins B6 and B12 and folic acid on homocysteine levels in hemodialysis patients and assess the functional impact of the methylenetetrahydrofolate reductase genotype on the response to treatment.

Methods: In a randomized prospective study, we assessed the effects of folic acid and two doses of B-vitamins in 50 hemodialysis patients with hyperhomocysteinemia. Patients were divided into two groups: 26 patients (group A) who received 25 mg of vitamin B6 daily and one monthly injection of 200 μg vitamin B12, and 24 patients (group B) who received 100 mg of vitamin B6 daily and one monthly injection of 1,000 μg vitamin B12. In addition, both groups received 15 mg folic acid daily. Patients were evaluated for homocysteine levels as well as for coagulation and a thorough lipid profile. Baseline Hcy levels were determined after at least 4 weeks washout from all folic acid and B-vitamins that were given. MFTHR alleles were analyzed, as were activated protein C resistance, von Willebrand factor and lupus anticoagulant.

Results: Basal plasma Hcy levels were significantly elevated in hemodialysis patients compared with normal subjects (33.8 ± 4.3 vs. 4.5 to 14.0 μmol/L). Following treatment, Hcy levels were significantly reduced to 21.2 ± 1.6 in group A and 18.6 ± 1.4 μmol/L in group B ($P < 0.01$). There was no difference in Hcy reduction following the administration of either high or low dosage of vitamins B6 and B12 utilized in the present study. There was no correlation between Hcy levels or thrombophilia and high incidence of thrombotic episodes in hemodialysis patients. Genotypic evaluation of MTHFR revealed that the presence of homozygous thermolabile MTHFR (n = 5) was associated with higher Hcy levels and better response to treatment (Hcy levels decreased by 58%, from 46.2 ± 14.6 to 19.48 ± 4.1 μmol/L following treatment). In patients with heterozygous thermolabile MTHFR (n = 25), Hcy levels decreased by 34%, from 31.2 ± 3.7 to 18.1 ± 1.1 μmol/L following treatment. The efficacy of high and low doses of B-vitamins on the reduction of homocysteine levels was comparable.

Conclusions: Treatment with B-vitamins in combination with folic acid significantly decreased homocysteine levels in hemodialysis patients, independently of the tested doses. In addition, mutations in MTHFR were associated with elevated plasma levels of Hcy. Neither vascular access nor the presence of diabetes was associated with higher pre- or post-treatment homocysteine level.

IMAJ 2004;6:213–217

The deleterious effects of severe hyperhomocysteinemia are seen in a wide range of cardiovascular diseases such as coronary heart, cerebrovascular, and peripheral vascular diseases [1–7]. Moreover, several studies have confirmed that moderate hyperhomocysteinemia is an independent predictor of the incidence of heart attack, and its reliability exceeds that of other risk factors such as smoking, hypertension, diabetes and dyslipidemia [1–7]. Since patients with end-stage renal disease have higher rates of myocardial infarction and cardiovascular diseases than the general population, the status of the homocysteine system in these patients has received much attention in the last few years [8,9]. Initially, Wilcken and Gupta [10] showed that plasma Hcy levels were elevated in chronic renal failure. Similar results were observed in ESRD patients on hemodialysis or peritoneal dialysis [2,11]. It is suspected, although not proven, that when moderately elevated, as in ESRD, hyperhomocysteinemia plays a central role in accelerating atherosclerosis and in creating a thrombophilic environment in the bloodstream [12–16].

The mechanisms underlying the elevated levels of Hcy are not fully known, although several explanations have been suggested [14–18]. For instance, it has been shown that renal tubuli metabolize Hcy; thus, loss of nephron mass may lead to hyperhomocysteinemia. Nevertheless, no significant mean renal arteriovenous differences for either total or non-protein-bound human Hcy have been proven [19]. In addition, the decline in glomerular filtration rate is strongly and independently associated with linear increase in fasting total Hcy levels.

The metabolism of Hcy requires two metabolic pathways, remethylation and trans-sulfuration. The remethylation of Hcy to methionine involves the acquisition of a methyl group from *N*-5-methyltetrahydrofolate. This reaction is mediated by MTHF reductase and is vitamin B12-dependent [2]. Therefore, it is widely accepted that deficiency of vitamins B12 and B6, as in patients with ESRD, or defects in the gene encoding MTHFR contribute to the high levels of Hcy in hemodialysis patients [2]. This notion has inspired several studies aimed at examining the effects of vitamins B6 and B12 and folate administration on the plasma levels of Hcy in different disease states, including atherosclerosis, renal transplant recipients, and hemodialysis patients. Such treatments lowered Hcy

*Deceased
Hcy = homocysteine
MTHFR = methylenetetrahydrofolate reductase

ESRD = end-stage renal disease

levels in predialysis patients by 30–50% and in renal transplant recipients by 25% after 6 weeks, with 75% achieving normalization of Hcy levels [20]. Similar beneficial results were obtained in patients with cystathionine β-synthase deficiency [20].

Most of the studies conducted in patients with ESRD were uncontrolled, and all of them failed to normalize Hcy levels. Recently, a randomized 8 week trial by Bostom et al. [14] revealed that administration of supraphysiologic doses of folic acid and vitamins B6 and B12 to hemodialysis patients reduced Hcy levels by 25–30% compared with control patients receiving placebo. However, in the latter study the authors utilized only one dose of each vitamin and did not refer to the MTHFR genotype of the studied patients. Therefore, the present study examined the effects of two doses of vitamins B6 and B12 and folic acid on Hcy levels in hemodialysis patients and addressed the functional impact of the MTHFR genotype on the response to treatment.

Patients and Methods

Our study was prospective and randomized. Fifty patients undergoing hemodialysis gave informed consent to participate in the study, which was approved by the institutional Helsinki Committee. After a 4 week washout period (for patients routinely receiving physiologic doses of folic acid), a preliminary pretreatment blood sample was taken. Following this baseline blood analysis, patients were divided into two groups: group A (n=24) and group B (n=26). Both groups received 15 mg of folic acid daily for 4 weeks; in addition, group A received vitamin B6 25 mg orally per day and a single subcutaneous injection of 200 μg vitamin B12 at the beginning of the 4 week treatment period, while group B received vitamin B6 100 mg orally per day and a single subcutaneous injection of 1,000 μg vitamin B12 at the beginning of the 4 week treatment period. No unusual side effects were observed. All patients were followed throughout the 4 weeks of treatment, after which a second blood sample was taken. Both blood samples were taken while the patients had been fasting for at least 8 hours beforehand. Healthy subjects served as controls. These subjects were of similar age to the hemodialysis patients (age 48 ± 11 years). Normal values of the examined biochemical parameters, except for Hcy, were provided by the clinical biochemistry laboratory at Rambam Medical Center.

No patients were receiving anticonvulsive drugs or chemotherapy with methotrexate. Liver functions and bilirubin were normal. Most of the patients were receiving erythropoietin. Additional parameters were tested, including lipid profile, coagulation profile, MTHFR genotype as described [21], lupus anticoagulant, fibrinogen, activated protein C resistance, and von Willebrand factor. Diabetic nephropathy and glomerulonephritis represent the main underlying diseases causing renal failure. Profile of the study participants are presented in Table 1.

Statistical analysis

Results are expressed as means ± SEM. Unpaired Student's *t*-test was used to check statistical differences between the different groups. $P < 0.05$ was considered statistically significant.

Table 1. Key baseline patient characteristics

	Normal values	Range in study	Mean in study
Age (yrs)	–	21–83	62
Time on dialysis (yrs)	–	–	3.26
Creatinine (mg/dl)	0.4–1.3	3.1–14.4	8.8
Urea (mg/dl)	5–20	27–121	65
Albumin (g/dl)	3.5–5.5	2.6-4.6	3.9
Total cholesterol (mg/dl)	150–230	91–281	180
High density lipoprotein (mg/dl)	30–80	20–72	37
Low density lipoprotein (mg/dl)	75–140	48–208	107
Triglycerides (μg/dl)	30–170	32–689	183
Hemoglobin (g/dl)	12–16	6.4–15.7	11.9
Hematocrit (%)	37–47	2—51	37
Calcium (mg/dl)	8.5–10.8	6.9–11.2	9.1
Homocysteine (μmol/L)	4.5–14.0	4.5–46.2	33.8
Folic acid (ng/ml)	3.1–12.4	5.9–16.8	6.1
Vitamin B12 (pg/ml)	223–1,132	394–470	433

Normal values of the examined biochemical parameters were provided by the clinical biochemistry laboratory at Rambam Medical Center.

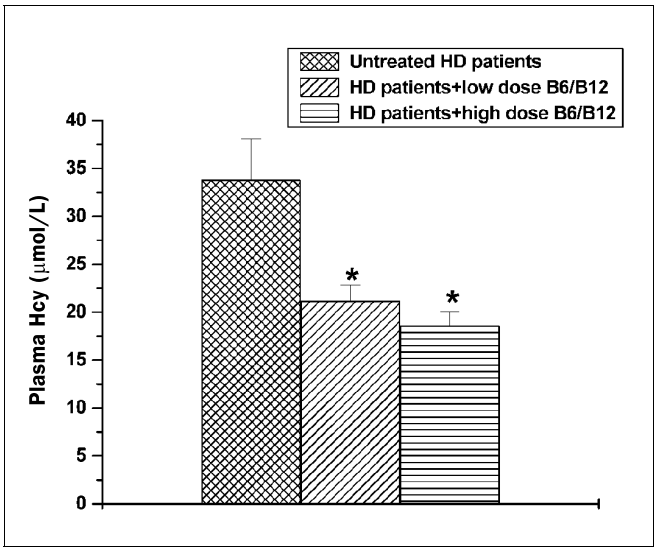

Figure 1. Effect of treatment with vitamin B on plasma homocysteine levels in hemodialysis patients (low doses in 24 patients and high doses in 25). Low dose = folic acid 15 mg/day + vitamin B6 25 mg/day + vitamin B12 200 μg/month; high dose = folic acid 15 mg/day + vitamin B6 100 mg/day + vitamin B12 1,000 μg/month.
*$P < 0.05$ compared with untreated hemodialysis (HD) patients.

Results

Analysis of dose-related response to treatment

In line with previous findings [2,17], patients on hemodialysis had higher Hcy levels compared with normal subjects (33.8 ± 4.3 vs. 9.8 ± 3.5 μmol/L, $P < 0.001$). After treatment, Hcy levels were significantly reduced in both groups ($P = 0.001$), but neither dose managed to normalize Hcy [Figure 1]. Hcy levels decreased by 42% in group A, from 31.8 ± 4.2 to 18.6 ± 1.4 μmol/L ($P < 0.001$) and by 41% in group B, from 36.0 ± 4.4 to 21.2 ± 1.6 μmolL ($P < 0.001$). These results are in agreement with similar studies conducted by Bostom et al. [14] and Sunder-Plassmann et al. [22]. In our study the mean Hcy level before treatment was 33.8 ± 4.3 μmol/L, and

the mean post-treatment level 19.9 μmol/L. Eighty-eight percent of our patients were above the 95th percentile of Hcy level as defined by Bostom et al. [14]. There was no significant difference between the declines in Hcy in response to the high and low doses of B-vitamins. The changes in Hcy levels were more profound with rising pretreatment values of Hcy, i.e., patients with higher levels of Hcy displayed better beneficial response to vitamin B treatment. In our study, the power of change in blood folic acid levels following treatment with both low and high doses of B-vitamins correlated directly with the extent of improvement of blood Hcy levels in studied patients.

Other parameters, hematologic or morbid states

No correlation was observed between age or gender and the beneficial response to vitamin treatment, nor was there a correlation between the responsiveness of our patients to vitamin B administration and their lipid profiles. Results of the coagulation profile of the studied patients are listed in Table 2. Of note was the fact that our hemodialysis patients displayed classic abnormal coagulation factors that were beyond the normal range. Fibrinogen was elevated in the pretreatment period and persisted after the administration of low and high doses of B-vitamins. Another remarkable observation was an elevated rattle venom test, which is a screening test for lupus anticoagulant. Von Willebrand factor was elevated in 25 of 48 patients. Activated protein C resistance was present in 48 patients.

Genotypic evaluation of MTHFR revealed that the presence of homozygous thermolabile MTHFR (n=5) was associated with higher Hcy levels and better response to treatment (Hcy levels decreased by 58%, from 46.2 ± 14.6 to 19.48 ± 4.06 μmol/L following treatment). In patients with heterozygous thermolabile MTHFR (n =25), Hcy levels decreased by 34%, from 31.2 ± 3.7 to 18.1 ± 1.1 μmol/L following treatment [Figure 2]. On the other hand, MTHFR genotype seemed to correlate with pretreatment folic acid values and response to treatment. The distribution of MTHFR alleles was as expected in the general population (10% homozygotes, 52% heterozygotes, 38% wild type).

Finally, concerning other morbid states, namely vascular access thrombosis and diabetes, results did not suggest a correlation (data not shown).

Discussion

In the present study, we assessed the effects of folic acid, 15 mg daily, and two doses of B-vitamins in 50 homodialysis patients with hyperhomocysteinemia. Our data clearly showed that Hcy levels were significantly reduced following treatment, but there was no difference in the reductions of Hcy concentrations following the administration of either low or high dosage of vitamin B6 or B12 used in the current study. There was no correlation between Hcy levels or thrombophilia and high incidence of thrombotic episodes in hemodialysis patients. Interestingly, hemodialysis patients with homozygous MTHFR genotype displayed higher basal levels of Hcy, and their response to B-vitamin treatment was more effective than that of heterozygous MTHFR hemodialysis patients and patients with moderate levels of Hcy.

Table 2. Selected coagulation parameters in study patients included in the study

	Normal values	Range in study	Mean value in study
Fibrinogen (mg/dl)	170–410	252–774	392
Protein C resistance (%)	>2	1.7–3.7	2.7
von Willebrand factor (%)	60–130	58–200	134
INR (%)	0.9–1.3	0.9–6.9	1.2
Platelets (per 10^3 MCRL)	130–400	8–374	181

Normal values of the examined biochemical parameters were provided by the clinical biochemistry laboratory at Rambam Medical Center.

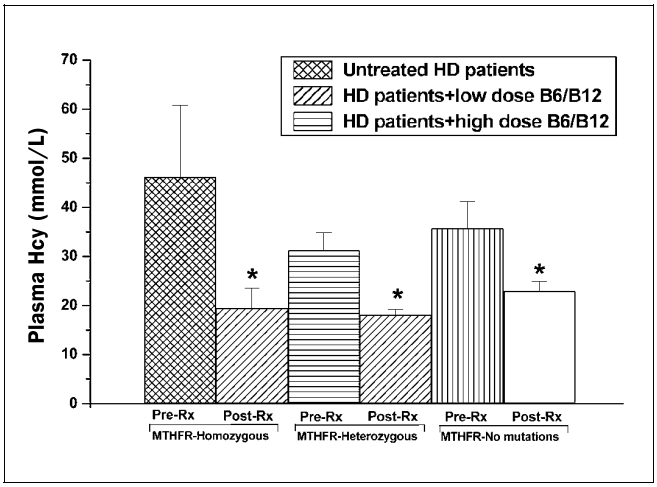

Figure 2. Effect of vitamin B treatment on plasma homocysteine levels in hemodialysis patients (low doses in 24 and high doses in 26) with different genotypes of MTHFR (5 patients homozygous and 25 heterozygous) and with no mutations (18 patients).
*$P < 0.05$ compared with basal plasma Hcy levels before treatment (Pre-Rx).

Although hemodialysis patients rarely present folate deficiency, an inverse correlation between serum folate and serum Hcy was demonstrated previously [2,23]. The former authors [2] reported that normal baseline serum folate concentrations are probably not sufficient to lower hyperhomocysteinemia in ESRD. Also, administration of 1 mg folic acid to dialysis patients is adequate to normalize plasma folate concentrations. However, such a dose has never been shown to normalize Hcy in dialysis patients. House and Donnelly [24] administered a standard multivitamin supplement containing 1 mg of folic acid to 11 chronic hemodialysis patients daily for 3 weeks and found that Hcy levels decreased by more than 20%. Dierkes et al. [25] recently reported the results of a study comparing 2.5 and 5 mg folic acid given orally to dialyisis patients and found that both supplementation regimens comparably reduced Hcy (Δ = 35%). We found that using a higher dose of folic acid, 15 mg daily by mouth, reduced Hcy to the same degree reported by those studies. While Bostom et al. [14] also administered 15 mg folic acid, the optimal dose of folic acid required to normalize serum Hcy levels remains unknown at this point. The rationale for giving supraphysiologic doses of folic acid is that it serves as a methyl donor in the remethylation reaction of Hcy metabolism and is consumed in that reaction. Sunder-Plassmann

and co-workers [22] studied the impact of two doses of folic acid, 30 and 60 mg per day, compared with 15 mg per day in an attempt to normalize hyperhomocysteinemia in 150 dialysis patients. The authors clearly demonstrated that high doses, i.e., 30 or 60 mg of folic acid per day, were not more effective than 15 mg per day in reducing hyperhomocysteinemia in regular dialysis patients. In addition, they found that patients with the MTHFR 677TT genotype benefited more than other dialysis patients in terms of normalizing total plasma Hcy levels. These findings clearly indicate that hyperhomocysteinemia in patients with ESRD cannot be cured solely by folic acid therapy, regardless of the dose, and that the efficacy of such a therapy depends on MTHFR genotype. However, Hcy is elevated in 35% of +/+ MTHFR individuals, and this rather "metabolic" relationship between a thermolabile MTHFR, which is only 40–60% active physiologically, and raised Hcy, makes so much sense. It is well established that the higher the level of Hcy in any given individuals, irrespective of whether they are on dialysis, the more efficient is vitamin treatment.

Besides folic acid, the impact of supplementation with other B-complex vitamins such as B6 and B12 is more difficult to assess. Several clinical trials investigating the impact of B-complex vitamin supplementation in hyperhomocysteinemic subjects found that the combination of folic acid, vitamin B6 and vitamin B12 may further reduce Hcy concentration when compared with isolated supplementation with these vitamins. Several studies have shown reductions in Hcy levels by 30–50% following various B-complex multivitamin regimens.

Manns et al. [8] studied the effects of various doses of a multivitamin regimen containing 1 mg folic acid per day on Hcy levels in 81 hyperhomocysteinemic hemodialysis patients. The patients were later switched to daily multivitamin therapy including 1 mg folic acid and 1 mg vitamin B12. Patients were then randomized to receive 4 weeks of either 5 or 20 mg folic acid in addition to the multivitamin and vitamin B12. According to these authors, the optimal oral treatment of hyperhomocysteinemia in dialysis patients consists of 1 mg folic acid and 1 mg oral vitamin B12 daily. However, they concluded that other effective therapies need to be developed, since only 13.6% of patients obtained normal Hcy levels with this treatment, and that further clinical trials are required to determine whether treatment with this regimen reduces the cardiovascular risk in ESRD patients.

It appears that hyperhomocysteinemia in hemodialysis patients may be improved by supraphysiologic doses of folic acid in combination with low physiologic doses of vitamin B6 or B12. Similar findings were observed by Bostom et al. [14] in a study of 27 dialysis patients divided into two groups, one receiving routine (physiologic) doses of vitamins (folic acid, vitamin B6 and vitamin B12) in addition to placebos and the second receiving supraphysiologic doses of all three vitamins in combination with the routine treatment. The lake of preliminary washout in that study [14] may have affected the mean starting Hcy levels that were relatively low (about 29.5 μmol/L), as was the improvement ($\Delta = -28.9\%$) after 4 weeks of treatment. These authors suggested that, while vitamins B6 and B12 played a role merely as co-factors, folic acid is actually a substrate in the Hcy catabolism pathway. It seems that a supraphysiologic dose of folic acid alone is sufficient to reduce plasma levels of Hcy, although the addition of treatment with physiologic doses of vitamins B6 and B12 may have a beneficial impact. Collectively, it is recommended not to exceed 5 mg/day of folate alone or in combination with 100 mg/day of B6 and a monthly injection of 200 mg of B12 in order to achieve optimal reduction in Hcy in hemodialysis patients. Such doses are safe and significantly lower than the dangerous and adverse doses of 200 mg B6, such as neurotoxicity and skin rash.

References

1. Van den Berg M, Boers GHJ. Homocystinuria: what about mild hyperhomocysteinaemia? *Postgrad Med J* 1996;72:513–18.
2. Bostom AG, Lathrop L. Hyperhomocysteinemia in end-stage renal disease: prevalence, etiology, and potential relationship to arteriosclerotic outcomes. *Kidney Int* 1997;52:10–20.
3. Sela BA, Doolman R. Dialysis patients and cyclosporine-treated graft recipients: two sectors with high homocysteine levels. *Harefuah* 1999;136(3):218–22 (Hebrew).
4. Zidek W. Homocysteine – a new arteriosclerotic risk factor in end-stage renal failure [Editorial]. *Nephron* 1997;75:249–50.
5. Welch GN, Loscalzo J. Homocysteine and atherothrombosis. *N Engl J Med* 1998;338:1042–50.
6. Bar-Meir E, George J, Shoenfeld Y. Hyperhomocysteinemia – a risk factor or vascular disease. *Harefuah* 1999;136(2):129–33 (Hebrew).
7. Sela B. Hyperhomocysteinemia as a risk factor in atherosclerotic and thrombotic vascular processes. *Harefuah* 1995;129(10):407–12 (Hebrew).
8. Manns B, Hyndman E, Burgess E, et al. Oral vitamin B_{12} and high-dose folic acid in hemodialysis patients with hyper-homocyst(e)inemia. *Kidney Int* 2001;59:1103–9.
9. Ma KW, Greene KL, Raij L. Cardiovascular risk factors in chronic renal failure and hemodialysis populations. *Am J Kidney Dis* 1992;19:505–13.
10. Wilcken DEL, Gupta VJ. Sulphr [sic] containing amino acids in chronic renal failure with particular reference to homocystine and cysteine-homocysteine mixed disulphide. *Eur J Clin Invest* 1979;9:301–7.
11. Maiorca R, Cancarini GC, Brunori G, Camerini C, Manili L. Morbidity and mortality of CAPD and hemodialysis. *Kidney Int* 1993;43(Suppl 40):S4–15.
12. Boushey CJ, Beresford SAA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. *JAMA* 1995;274:1049–57.
13. Selhub J, Jacques PF, Bostom AG, et al. Association between plasma homocysteine concentrations and extracranial carotid-artery stenosis. *N Engl J Med* 1995;332:286–91.
14. Bostom AG, Shemin D, Lapane KL, et al. High dose B-vitamin treatment of hyperhomocysteinemia in dialysis patients. *Kidney Int* 1996 49:147–52.
15. Robinson K, Gupta A, Dennis V, et al. Hyperhomocysteinemia confers an independent increased risk of atherosclerosis in end-stage renal disease and is closely linked to plasma folate and pyridoxine concentrations. *Circulation* 1996;94:2743–8.
16. Ridker PM, Hennekens CH, Selhub J, Miletich JP, Malinow MR, Stampfer MJ. Interrelation of hyperhomocysteinemia, factor V Leiden, and risk of future venous thromboembolism. *Circulation* 1997;95:1777–82.
17. Wilcken DEL, Dudman NPB, Tyrrell PA, Robertson MR. Folic acid lowers elevated plasma homocysteine in chronic renal insufficiency: possible implications for prevention of vascular disease. *Metabolism* 1988;37:697–701.
18. Dennis VW, Robinson K. Homocysteinemia and vascular disease in end-stage renal disease. *Kidney Int* 1996;50(Suppl 57):S11–17.
19. Van Guldener C, Donker AJM, Jakobs C, Teerlink T, de Meer K, Stehouwer CDA. No net renal extraction of homocysteine in fasting humans. *Kidney Int* 1998;54:166–99.

20. Bostom AG, Culleton B. Hyperhomocysteinemia in chronic renal disease: potential relevance to arteriosclerosis. *Semin Dial* 1999;12: 103–11.
21. Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 1995;10:111–13.
22. Sunder-Plassmann G, Födinger M, Buchmayer H, et al. Effect of high dose folic acid therapy on hyperhomocysteinemia in hemodialysis patients: results of the Vienna Multicenter Study. *J Am Soc Nephrol* 2000;11:1106–16.
23. Bostom AG, Shemin D, Lapane KL, et al. Folate status is the major determinant of fasting total plasma homocysteine levels in maintenance dialysis patients. *Atherosclerosis* 1996;123:193–202.
24. House AA, Donnelly JG. Effect of multivitamins on plasma homocysteine and folate levels in patients on hemodialysis. *ASAIO J* 1999;45:94–7.
25. Dierkes J, Domrose U, Ambrosch A, Bosselmann HP, Neumann KH, Luley C. Response of hyperhomocysteinemia to folic acid supplementation in patients with end-stage renal disease. *Clin Nephrol* 1999;51:108–15.

Correspondence: Dr. F. Nakhoul, Dept. of Nephrology, Rambam Medical Center, P.O. Box 9602, Haifa 31096, Israel.
Phone: (972-4) 854-2852
Fax: (972-4) 854-2946
email: f_nakhoul@rambam.health.gov.il