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

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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. MTHFR 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 49.2 ± 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.

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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, Wilden 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 N5-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 folic 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

IMA 04:6:213-217

Homocysteine in Hemodialysis Patients 213
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

<table>
<thead>
<tr>
<th></th>
<th>Normal values</th>
<th>Range in study</th>
<th>Mean in study</th>
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<tbody>
<tr>
<td>Age (yrs)</td>
<td>–</td>
<td>21–83</td>
<td>62</td>
</tr>
<tr>
<td>Time on dialysis (yrs)</td>
<td>–</td>
<td>–</td>
<td>3.26</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.4–1.3</td>
<td>3.1–14.4</td>
<td>8.8</td>
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<tr>
<td>Urea (mg/dl)</td>
<td>5–20</td>
<td>27–121</td>
<td>65</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.5–5.5</td>
<td>2.6–4.6</td>
<td>3.9</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>150–230</td>
<td>91–218</td>
<td>180</td>
</tr>
<tr>
<td>High density lipoprotein (mg/dl)</td>
<td>30–80</td>
<td>20–72</td>
<td>37</td>
</tr>
<tr>
<td>Low density lipoprotein (mg/dl)</td>
<td>75–140</td>
<td>48–208</td>
<td>107</td>
</tr>
<tr>
<td>Triglycerides (μg/dl)</td>
<td>30–170</td>
<td>32–689</td>
<td>188</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>12–16</td>
<td>6.4–15.7</td>
<td>11.9</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>37–47</td>
<td>2–51</td>
<td>37</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>8.5–10.8</td>
<td>6.9–11.2</td>
<td>9.1</td>
</tr>
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<td>Homocysteine (μmol/L)</td>
<td>4.5–14.0</td>
<td>4.5–46.2</td>
<td>33.8</td>
</tr>
<tr>
<td>Folic acid (mg/ml)</td>
<td>3.1–12.4</td>
<td>5.9–16.8</td>
<td>6.1</td>
</tr>
<tr>
<td>Vitamin B12 (μg/ml)</td>
<td>223–1,132</td>
<td>394–470</td>
<td>433</td>
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</table>

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 μmol/L (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 homozgyous thermolabile MTHFR (n=5) was associated with higher Hcy levels and better response to treatment (Hcy levels decreased by 58%, from 462 ± 146 to 1948 ± 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 13.1 ± 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 hemodialysis 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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal values</th>
<th>Range in study</th>
<th>Mean value in study</th>
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<tbody>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>170-610</td>
<td>252-774</td>
<td>392</td>
</tr>
<tr>
<td>Protein C resistance (%)</td>
<td>&gt;2</td>
<td>1.7-3.7</td>
<td>2.7</td>
</tr>
<tr>
<td>von Willebrand factor (%)</td>
<td>60-130</td>
<td>58-200</td>
<td>134</td>
</tr>
<tr>
<td>INR (%)</td>
<td>0.9-1.3</td>
<td>0.9-6.9</td>
<td>1.2</td>
</tr>
<tr>
<td>Platelets (per 10^3 μmol/L)</td>
<td>130-600</td>
<td>8-374</td>
<td>181</td>
</tr>
</tbody>
</table>

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).](image-url)

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 25 and 5 mg folic acid given orally to dialysis 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 thermostable 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 placebo and the second receiving supraphysiologic doses of all three vitamins in combination with the routine treatment. The lack 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 (∆ = –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


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**Capsule**

**Calcium and the developing brain**

Calcium signaling communicates many effects of activity in the neural system. Short-term effects may be mediated by protein modifications, whereas long-term effects may be mediated by changes in gene transcription. Aizawa et al. have identified a calcium-responsive protein called CREST that is a transcriptional transactivator. Embryonic development was unaffected in mice lacking CREST, but these mice displayed severe defects in cortical and hippocampal dendrite development. Cortical neurons from mice lacking CREST showed deficiencies in dendritic growth in response to depolarization. Thus, the effects of electrophysiologic activity on gene expression and subsequent neuronal development may be mediated by CREST. *Science* 2004;303:97

E. Israeli

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**Capsule**

**Vaccines in the spotlight**

Although the clinical and socioeconomic effect of influenza may vary from year to year, the burden of disease in children always represents a substantial public-health problem. Should all children be vaccinated against influenza to avoid the excess hospitalizations, medical visits, and antibiotic use that recent studies suggest? Some experts already encourage influenza vaccination in childhood on this basis. Principi and Esposito advocate wider use of the influenza vaccine, especially for children aged 6–23 months, those with recurrent acute otitis media or respiratory tract infections, and those attending daycare centers. At present, licensed vaccines are safe, well tolerated, and highly effective, but until economic data are fully in accordance and universal vaccination may be some time off. Immunization is again in the spotlight, when Tom Jefferson and colleagues reviewed evidence of adverse events after exposure to aluminium-containing vaccines against diphtheria, tetanus and pertussis. In a meta-analysis of data from five studies the authors compared vaccines containing aluminium hydroxide versus no adjuvant, and in the age groups up to 18 months and 10–16 years. Finding no evidence that aluminium salts in vaccines cause any serious or long-lasting events, the authors recommend, unusually, that no further research on this topic be undertaken. *Lancet Infect Dis* 2004 (online)