Kidney International, Vol. 58 (2000), pp. 851–858

Hyperhomocysteinemia in hemodialysis patients: Effects of 12-month supplementation with hydrosoluble vitamins

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Hyperhomocysteinemia in hemodialysis patients: Effects of 12-month supplementation with hydrosoluble vitamins.

Background. High-efficiency hemodialysis may induce a deficiency in hydrosoluble vitamins. Supplementation with B-complex vitamins has been shown to lower serum homocysteine concentrations in several groups, but relatively few studies have concerned hemodialysis patients. Our objectives were to determine the status in B-complex vitamins in a large cohort of unsupplemented hemodialysis patients and to assess the effects of supplementation with hydrosoluble vitamins on serum homocysteine over one year.

Methods. Serum total homocysteine (tHcy), vitamin B_{12}, folate, pyridoxal-5′-phosphate (P-5′-P; the active moiety of vitamin B_6), as well as red blood cell folate concentrations, were measured in 168 chronic dialysis patients on three times weekly high-efficiency hemodialysis and not supplemented with hydrosoluble vitamins. Their methylenetetrahydrofolate reductase C677T (MTHFR) genotypes were also determined (homozygotes TT, heterozygotes CT, without mutation CC). All involved patients were then supplemented with hydrosoluble vitamins (once daily by mouth, DiaVite®; R&D Laboratories, Minneapolis, MN, USA), and half of them were randomized to receive in addition 10 mg intravenously of folic acid posthemodialysis (30 mg intravenously per week). Serum tHcy was monitored after 6 and 12 months of supplementation in the 140 and 128 patients available for follow-up.

Results. At baseline, serum and red blood cell folate concentrations were within normal limits in all patients except for two with borderline serum folate (mean values of 21 ± 8 and 1195 ± 454 nmol/L), whereas serum vitamin B_{12} and P-5′-P were below normal in 11 and 65 patients, respectively (mean values of 327 ± 215 pmol/L and 19 ± 16 nmol/L for the 168 patients). Initial tHcy levels were increased in all patients (mean 33.3 ± 16.6 for a normal below 11.8 ± 1.5 μmol/L); tHcy significantly decreased to 23.5 ± 7.6 μmol/L after six months ($P < 0.0001$ vs. baseline) and to 21.7 ± 6.1 μmol/L after 12 months ($P < 0.0001$ vs. baseline) for the entire group, but was normalized in only four patients at 12 months. After six months, the mean reduction in tHcy was slightly but significantly greater for patients receiving intravenous folic acid (12.2 ± 18.5 μmol/L) compared with patients not receiving it (8.3 ± 9.8 μmol/L, $P < 0.05$). However, at 12 months, no difference between both subgroups persisted. When considering the different genotypes, tHcy at baseline tended to be higher for TT than CT and CC (39.8 ± 30.9 vs. 31.4 ± 10.5 vs. 31.6 ± 11.8 μmol/L) and decreased to respective values of 21.1 ± 6.9 versus 21.4 ± 6.1 versus 22.2 ± 5.9 μmol/L at 12 months. The impact of the addition of folic acid to DiaVite® appeared particularly significant in TT patients at six months.

Conclusions. (*1*) Hyperhomocysteinemia was present in 100% of our hemodialysis patients. (*2*) Nearly 40% of our unsupplemented hemodialysis patients were deficient in vitamin B_6. (*3*) Supplementation with DiaVite® has resulted in significant tHcy reductions for all three genotypes. (*4*) The impact of the proposed supplementation protocol was found after six months and was maintained, but did not increase further after 12 months of the same regimen. (*5*) The addition of intravenous folic acid has been associated with a more pronounced decrease in tHcy in TT patients.

Hyperhomocysteinemia is now recognized as an independent risk factor for atherosclerotic cardiovascular diseases in the general population [1–5]. There is a higher prevalence of hyperhomocysteinemia in end-stage renal disease (ESRD) patients who are predialysis and on dialysis [6, 7], as well as in renal transplant patients [8, 9]. In both populations, the increased incidence of atherosclerotic vascular disease has been shown to correlate with the level of serum homocysteine [9–13]. Moreover, ESRD patients have serum total homocysteine (tHcy) values twofold to threefold higher than those of age-matched controls with normal renal function [14, 15].

Recognized causes of hyperhomocysteinemia include deficiencies or mutation of enzymes involved in the metabolism of methionine, deficiencies in various cofactors involved in the metabolism of homocysteine, and renal insufficiency. Serum homocysteine can be lowered with supplementation of cofactors involved in its metabolism. Hence, supplementation with folate, vitamins B_{12} and B_6,

Key words: homocysteine, folate, vitamin B_{12}, pyridoxal-5′-phosphate, atherosclerotic cardiovascular disease, end-stage renal disease.

Received for publication August 12, 1999
and in revised form January 25, 2000
Accepted for publication March 10, 2000

alone or in combination, has been successful in reducing homocysteine concentrations to various degrees in different subgroups of patients [16–22]. Although a reduction in serum homocysteine has been associated with fewer atherothrombotic events in patients afflicted by the genetic disorder homocystinuria [23, 24], its benefit remains to be proven for milder degrees of hyperhomocysteinemia such as that which occurs in chronic renal failure patients.

On the other hand, patients on hemodialysis are at risk for deficiency in hydrosoluble vitamins as a consequence of either poor intake, decreased absorption, altered metabolism, and/or dialysis-induced losses [25–27]. High-flux hemodialysis may further decrease serum concentrations of hydrosoluble vitamins [27, 28], while erythropoietin therapy may precipitate folate and vitamin B_{12} deficiencies [29, 30]. Most of the studies that have addressed the impact of vitamin supplementation on homocysteine levels in ESRD patients were performed over short time periods [31–40], with only one recent study reporting results over one year [41]. Moreover, chronic renal disease is associated with a relative resistance to the lowering effects of low-dose folate supplementation on homocysteine [36, 42]. Whereas 1 to 5 mg/day of folic acid may normalize plasma homocysteine in moderately hyperhomocysteinemic individuals with normal renal function, a similar effect has not been observed in ESRD patients. Hence, the optimal dose as well as the ultimate benefits remain to be clearly demonstrated in chronic dialysis patients.

The purpose of the present study was to assess the effects of enteral (± parenteral) supplementation with hydrosoluble vitamins on serum homocysteine concentrations in a large cohort of chronic hemodialysis patients over a one year interval. Concomitantly, the methylenetetrahydrofolate reductase (MTHFR) genotype of our group of patients was determined, since a mutation of this enzyme is commonly related to hyperhomocysteinemia [43]. This allowed us to evaluate the impact of supplementation with hydrosoluble vitamins for the different genotypes of this enzyme prevailing in our hemodialysis population.

METHODS

Patients and hemodialysis technique

One hundred sixty-eight chronic dialysis patients were initially enrolled in the study after informed consent. All patients were on hemodialysis at the beginning of the study in May 1998, with a regimen including three times weekly sessions (3.5 to 4 hours each) using high-efficiency reused dialyzers (CT190, Dicea170, Tricea210; Baxter, McGaw Park, IL, USA; or F8, F80; Fresenius, Bad Homburg, Germany; or Filtral 20, Hospal, Lyon, France) at a blood flow rate of 350 to 450 mL/min and a dialysate flow rate of 500 to 600 mL/min.

Blood sampling and vitamin supplementation

None of the involved patients were receiving vitamin supplementation at study entry, since it had not been a routine practice in our unit for more than five years. At the beginning of the study, baseline values of serum tHcy, serum vitamin B_{12}, serum folate, serum pyridoxal-5′-phosphate (P-5′-P, the active moiety of vitamin B_6), and red blood cell folate were measured in all patients.

Thereafter, all patients were supplemented with hydrosoluble vitamins orally (DiaVite® once daily taken by mouth; R&D Laboratories, Marina del Rey, CA, USA; note that Nephro-Vite® is marketed in Canada as DiaVite®). Moreover, half of the patients were randomized to receive an additional 10 mg of folic acid intravenously posthemodialysis, and hence were administered 30 mg intravenously per week. Measurements of serum tHcy, folate, vitamin B_{12}, and P-5′-P were repeated after 6 and 12 months of supplementation. The MTHFR genotype status of our patients was also determined.

DiaVite® is a hydrosoluble multivitamin formulation containing per capsule 1 mg of folic acid, 10 mg of pyridoxine (vitamin B_6), and 6 μg of cobalamin (vitamin B_{12}). It also contains ascorbic acid (vitamin C, 60 mg), thiamine (vitamin B_1, 1.5 mg), riboflavin (vitamin B_2, 1.7 mg), nicotinic acid (20 mg), panthothenic acid (10 mg), and biotin (300 μg).

Homocysteine assay

Total homocysteine serum concentrations (comprising both free and protein-bound forms) were obtained predialysis. After drawing, blood was placed immediately on ice, and serum was separated promptly by centrifugation at 3000 r.p.m. The serum fraction was removed and then stored at −80°C until assayed. Total serum homocysteine levels were measured using high-performance liquid chromatography (HPLC) with fluorometric detection as recently described [44]. This method allows the simultaneous measurement of other thiols found in serum (cysteine, cysteinylglycine, and glutathion). Normal values are below 11.8 ± 1.5 μmol/L for males and below 10.9 ± 2.0 μmol/L for females.

Serum measures of B-complex vitamins

Serum folate and vitamin B_{12} were determined by immunochemiluminescence (Access Immunoassay Instruments, Beckman, Mississauga, Canada). Normal values for folate and vitamin B_{12} were >10 nmol/L and 190 to 730 pmol/L, respectively, whereas values compatible with frank deficiencies are <7 nmol/L and <150 pmol/L, respectively. P-5′-P, the active moiety of vitamin B_6, was measured by radioenzymatic assay (vitamin B_6 ^{3}H REA; Bühlmann Laboratories, American Laboratory Products,

Table 1. Baseline characteristics of patients randomized for IV folic acid

	Folic acid IV	No folic acid IV
Number	83	85
Age *years*	60.2 ± 14.1	61.4 ± 13.8
Sex ratio *male/female*	50/33	49/36
Diabetes *N of patients*	24	26
Causes of ESRD		
Diabetes	18	12
Glomerular diseases	16	16
Ischemic renal diseases	11	14
Hypertensive nephrosclerosis	12	15
Chronic pyelonephritis	4	6
Polycystic disease	9	5
Other	5	12
Unknown	8	5
Time on dialysis *year*	3.4 ± 4.6	3.0 ± 3.8
Mean single pool *Kt/V*	1.64 ± 0.30	1.72 ± 0.37
Mean serum albumin *g/L*	38.4 ± 4.4	38.2 ± 5.4
Vascular disease *N of patients*	47	46
History of access thrombosis		
N of patients	18	22
Active smoking *N of patients*	41	22

No difference in both subgroups except for the prevalence of active smoking ($P = 0.02$).

Table 2. Concentrations of B-complex vitamins and homocysteine over the study period for all patients, and by subgroups with or without folic acid

	Baseline	6 months	12 months
RBC folate *nmol/L*			
All	1195.1 ± 454.0		
+Folic acid	1156.6 ± 417.2		
−Folic acid	1235.7 ± 488.9		
Serum folate *nmol/L*			
All	20.84 ± 7.62		
+Folic acid	20.43 ± 7.34		all > 45[a]
−Folic acid	21.26 ± 7.93		43.41 ± 5.13[a]
Vitamin B_{12} *pmol/L*			
All	326.9 ± 215.2		493.7 ± 488.4[a]
+Folic acid	319.3 ± 228.8		531.4 ± 650.9[a]
−Folic acid	334.6 ± 202.0		449.6 ± 149.2[a]
P-5′-P *nmol/L*			
All	19.1 ± 15.6		128.8 ± 97.4[a]
+Folic acid	18.2 ± 15.2		125.2 ± 99.5[a]
−Folic acid	20.0 ± 16.1		129.1 ± 95.8[a]
tHcy *μmol/L*			
All	33.27 ± 16.59	23.54 ± 7.56[a]	21.69 ± 6.12[a]
+Folic acid	34.75 ± 19.96	22.66 ± 7.60[ab]	21.69 ± 6.94[a]
−Folic acid	31.83 ± 12.39	24.62 ± 7.48[a]	21.69 ± 5.00[a]

Abbreviations are: RBC, red blood cells; P-5′-P, pyridoxal-5′-phosphate; tHcy, total homocysteine.
[a] $P < 0.0001$ vs. baseline
[b] $P < 0.05$ for +folic acid vs. −folic acid

Windham, NH, USA); normal values for P-5′-P were between 11.5 and 179.3 nmol/L (as reported by the manufacturer). Red blood cell folate was obtained using the same method as for serum folate but applied after cell lysis. Normal values were between 375 and 2500 nmol/L, and values compatible with deficiency were <125 nmol/L.

MTHFR genotype determination

The polymerase chain reaction primers for the amplification of the MTHFR mutation have been described elsewhere [45]. If the MTHFR mutation is present, *Hin*fI digests the 198 bp fragment into a 175 bp and a 23 bp fragments. Analyzing the fragments in 2.5% polyacrylamide gel electrophoresis allows the determination of the length of the fragments.

In the next section, results are presented as mean ± SD. Statistics applied were analysis of variance or paired or unpaired Student's *t*-test, where appropriate.

RESULTS

At baseline, there were 99 males and 69 females with a mean age of 60.8 ± 7.2 years; they had been on hemodialysis for an average of 3.2 ± 0.9 years. In the preceding months, their mean Kt/V and nPCR (normalized protein catabolic rate) were, respectively, 1.68 ± 0.06 and 0.95 ± 0.17 g/kg/day. Causes of ESRD are shown in Table 1. Demographic parameters for the two subgroups following randomization for intravenous folic acid are also presented in Table 1.

After 6 months, 28 patients (17%) were lost at follow-up for the following reasons: death ($N = 12$), switch to peritoneal dialysis (7), renal transplantation (7), and transfer to another dialysis center (2). At 12 months, 12 more patients (7%) were lost at follow-up as a consequence of death (7), switch to peritoneal dialysis (2), renal transplantation (2), and transfer to another center (1). None were withdrawn from the study for side effects of vitamin supplementation. All patients were included in the baseline analysis, and therefore, 140 patients remained in the analysis at 6 months and 128 patients at 12 months.

Table 2 shows the evolution of serum folate, vitamin B_{12}, P-5′-P, tHcy, and red blood cell folate concentrations over the study period in all patients involved in the study and by subgroups receiving or not receiving folic acid. At baseline, serum and red blood cell folate levels were within normal limits in all unsupplemented patients, except for two patients having borderline serum folate (above 7 but below 10 nmol/L). Although the mean value of serum vitamin B_{12} was also within normal limits (326.9 ± 215.2 pmol/L), 11 patients (6%) were deficient, having values below 150 pmol/L at baseline. P-5′-P was below normal in a large proportion of patients at the start (65 patients or 39%), with initial mean and median values of 19.1 ± 15.6 and 16.7 nmol/L for the entire group. Figure 1 shows the distribution of serum P-5′-P in our unsupplemented patients at study entry. After 12 months of supplementation with DiaVite®, the median was increased to 106.6 nmol/L, and only 5 patients had P-5′-P values below the lower normal limit (suggesting either poor absorption and/or compliance).

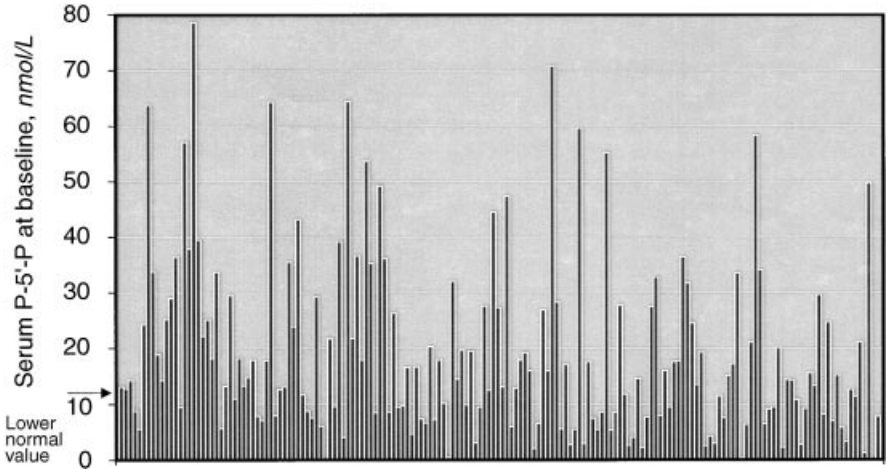

Fig. 1. Distribution of serum pyridoxal-5′-phosphate (P-5′-P) concentrations (nmol/L) in the 168 unsupplemented hemodialysis patients at study entry (one bar per patient).

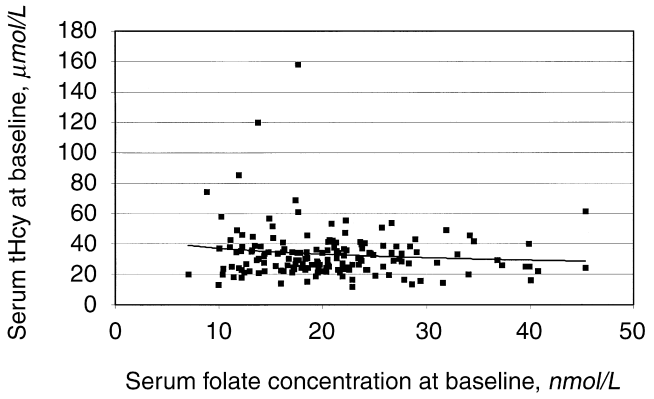

Fig. 2. No significant relation was observed between serum total homocysteine (tHcy; μmol/L) and serum folate (nmol/L) concentrations in our unsupplemented hemodialysis patients at baseline. y = −5.6 Ln(x) + 50.0; R^2 = 0.014.

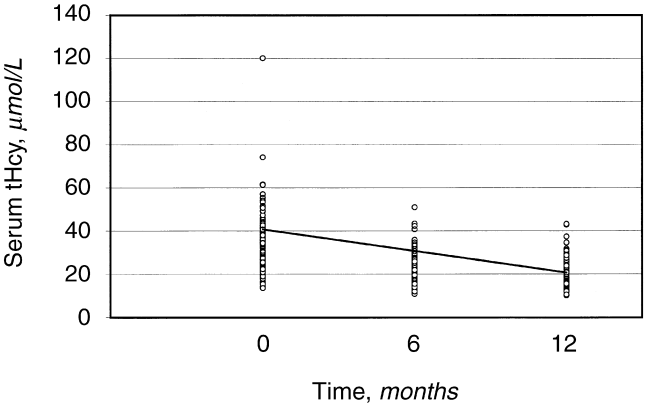

Fig. 3. Evolution of total serum homocysteine (μmol/L) over 12 months of the described vitamin regimen (DiaVite® 1 daily by mouth ± folic acid intravenously 30 mg per week) in the 128 patients available at follow-up. y = −10.0x + 50.7; R^2 = 0.58; $P < 0.0001$.

At baseline, tHcy serum concentrations were above normal values in all our patients (100%), ranging from 12.01 to 158 μmol/L (mean of 33.3 ± 16.6 μmol/L and median of 29.7 μmol/L). Despite an observed tendency, there was no significant negative correlation between initial tHcy and serum folate concentrations in our unsupplemented hemodialysis patients (Fig. 2). After 6 months of supplementation with DiaVite®, tHcy serum concentrations decreased significantly to a mean of 23.5 ± 7.6 μmol/L for the remaining 140 patients ($P <$ 0.0001). In addition, the mean reduction in tHcy tended to be higher in the subgroup also receiving intravenous folic acid, but the difference did not reach significance. We found a mean reduction of tHcy of 12.2 ± 18.5 μmol/L for those supplemented with intravenous folic acid as a group compared with 8.1 ± 9.7 μmol/L for those without the intravenous supplementation at six months ($P < 0.05$). At 12 months, no further difference in tHcy between both subgroups was found. However, serum tHcy was normalized in only four patients at 6 months and in four patients at 12 months (only 1 patient included at both times), of whom seven (over 8) were receiving intravenous folic acid. There was no further significant decrease in tHcy at 12 months (with or without folic acid). Figure 3 presents serial tHcy serum concentrations (at time 0, 6, and 12 months) for the 128 patients remaining in the study at 12 months.

The distribution of MTHFR genotypes obtained for 161 of the involved hemodialysis patients was as follows: 68 patients (42%) had no mutation (CC); 67 (42%) were heterozygotes (CT), and 26 (16%) were homozygotes (TT). Among CC, 35 were randomized to intravenous folic acid, among CT, 34, and finally, among TT, 13 patients. Baseline tHcy, as well as changes in tHcy values, was found to be slightly different in the subgroup analysis by genotypes. Table 3 shows tHcy values at baseline, at 6, and at 12 months for each MTHFR genotype (for patients remaining in the study at 12 months). Hence,

Table 3. Evolution of total homocysteine (tHcy) for the different MTHFR genotypes

	Baseline	6 months	12 months
No mutation (CC)			
All	31.65 ± 11.79	24.64 ± 7.93[a]	22.22 ± 5.92[a]
+Folic acid 34	34.02 ± 12.35	24.73 ± 8.32[ab]	22.64 ± 6.94[a]
−Folic acid 28	30.47 ± 12.10	24.85 ± 7.40[a]	21.69 ± 4.01[a]
Heterozygotes (CT)			
All	31.38 ± 10.46	22.27 ± 7.29[a]	21.36 ± 6.13[a]
+Folic acid 28	31.58 ± 9.83	21.49 ± 5.71[ab]	20.94 ± 6.16[a]
−Folic acid 31	32.42 ± 12.03	23.13 ± 8.45[a]	21.85 ± 6.07[a]
Homozygotes (TT)			
All	39.79 ± 30.89	23.45 ± 7.13[a]	21.06 ± 6.89[a]
+Folic acid 11	51.55 ± 31.83	21.82 ± 8.86[ac]	22.60 ± 9.06[a]
−Folic acid 12	32.20 ± 6.08	24.94 ± 5.04[a]	19.80 ± 4.51[a]

[a] $P < 0.0001$ vs. baseline
[b] $P < 0.05$ vs. −folic acid
[c] $P < 0.01$ vs. −folic acid

mean tHcy values at baseline tended to be higher for homozygotes (TT, 39.8 ± 30.9 μmol/L) than for heterozygotes (CT, 31.4 ± 10.5 μmol/L) or patients without the mutation (CC, 31.6 ± 11.8 μmol/L). Subsequent analysis at six months showed diminution of tHcy levels to 23.4 ± 7.1 versus 22.3 ± 7.3 versus 24.6 ± 7.9 μmol/L, respectively. At 12 months, tHcy values for respective genotypes (21.1 ± 6.9 vs. 21.4 ± 6.1 vs. 22.2 ± 5.9 μmol/L) were similar to those found at six months. The evolution of tHcy is also presented according to the two arms of the study, with and without intravenous folic acid. The addition of folic acid to DiaVite® did result in a further decrease, although small, in tHcy at six months, particularly in homozygotes for MTHFR in whom the most significant impact was observed ($P = 0.036$; Fig. 4).

DISCUSSION

Dialysis patients are at increased risk for deficiencies in hydrosoluble vitamins [25, 46], since hemodialysis is associated with a relatively high removal rate of water-soluble vitamins. Potential losses are even more pronounced in patients undergoing high-flux or high-efficiency hemodialysis, as shown recently by Kasama et al for vitamin B_6 [27]. Our results confirm that a significant proportion of chronic hemodialysis patients are deficient in vitamin B_6 if not supplemented (65 patients, or nearly 40%, with overt deficiencies and 61 more patients, or 36%, with borderline values, defined as between 11.5 and 25.5 nmol/L). A normal folate status was found in almost all of our unsupplemented patients despite substantial folate losses during hemodialysis; however, a functional deficit was not excluded. Serum vitamin B_{12} was normal in all but five patients at baseline. These results are similar to those of Descombes, Hanck, and Fellay, who found a vitamin B_6 deficiency in up to 89% of unsupplemented patients, and normal serum folate and vitamin B_{12} concentrations in dialysis patients receiving only folic acid supplementation [26], as well as the work of Robinson et al, who showed that deficiency in vitamin B_6 was more frequent in ESRD patients than in the normal reference population [47]. The present study shows that 10 mg daily of pyridoxine normalizes P-5′-P serum concentrations in a large group of chronic hemodialysis patients, and this highlights the increased requirements in pyridoxine of such a population.

Although hemodialysis patients rarely present folate deficiency, an inverse correlation between serum folate and serum homocysteine has been demonstrated previously [48]. Only a tendency, and no significant inverse correlation, was observed in our unsupplemented patients at enrollment in the study. Nonetheless, it has been proposed that these "normal" baseline serum folate concentrations probably do not suffice for lowering hyperhomocysteinemia in ESRD [34]. Therefore, investigators have begun to give supraphysiologic doses of folic acid in an attempt to decrease homocysteine levels [17, 21, 22]. In hemodialysis patients, a dose of 1 mg daily by mouth of folic acid has been shown sufficient to normalize plasma folate concentrations [26] and thus has been advocated; however, such a dose has never been shown to normalize homocysteine in ESRD patients [25]. House and Donnelly gave a standard multivitamin supplement daily (Nephro-Vite® marketed in Canada as DiaVite® and containing 1 mg of folic acid) to 11 chronic hemodialysis patients during three weeks [49]; homocysteine levels decreased by more than 20%. Dierkes et al recently reported the results of a study comparing 2.5 to 5 mg of folic acid by mouth in hemodialysis and peritoneal dialysis patients [50]; they found that both supplementation regimens reduced homocysteine to the same degree, by nearly 35%. Van Guldener et al measured homocysteine concentrations in 26 hemodialysis patients randomized to receive either 1 or 5 mg daily by mouth of folic acid after 52 weeks [41]; at the end of the study, homocysteine levels did not differ significantly between both groups.

The rationale for giving supraphysiologic doses of folic acid is that it serves as a methyl donor in the remethylation reaction of homocysteine metabolism and is "consumed" in that reaction. Therefore, by providing a greater amount of folic acid, one can further lower homocysteine levels towards normal. Bostom et al have given as much as 15 mg/day of folic acid without side effects [33]. However, the optimal dose of folic acid required to normalize serum homocysteine, or else, that provides the maximal impact on hyperhomocysteinemia remains unknown at this point. During the study, our patients were all supplemented with an enteral mixture of hydrosoluble vitamins in the form of DiaVite®, containing 1 mg of folic acid. Since the content of DiaVite® in folic acid is relatively small, it was decided to randomize patients to receive an additional amount of 10 mg intravenous

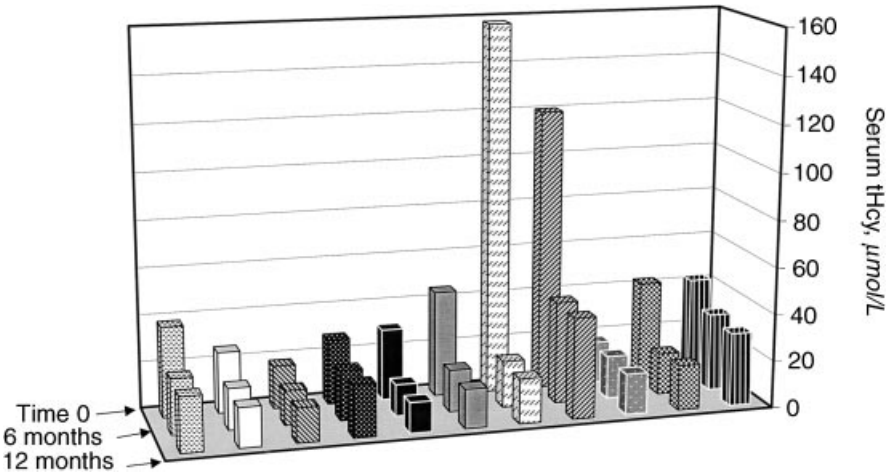

Fig. 4. Serum total homocysteine (tHcy; μmol/L) over 12 months for hemodialysis patients homozygous for MTHFR who received both oral DiaVite® and intravenous folic acid. Eleven patients were available for the entire follow-up. Each patient is represented with a different bar type.

of folic acid at each dialysis session (or 30 mg/week). Such a regimen was considered practical from a clinical trial standpoint. After one year of this regimen, respective reductions of 37.5 and 31.8% in serum tHcy concentrations compared with baseline values were observed in patients receiving and not receiving intravenous folic acid. Therefore, although present, the effect of the addition of folic acid to DiaVite® does not seem major.

Besides folic acid, the impact of a supplementation with the other B-complex vitamins such as B_6 and B_{12} is more difficult to assess. Several clinical trials looking at the impact of B-complex vitamin supplementation in hyperhomocysteinemic subjects have found that combining folic acid, vitamin B_6, and vitamin B_{12} may reduce further the homocysteine concentration when compared with isolated supplementation with folic acid, B_6, or B_{12} [17, 20, 21]. The impact of vitamin B_6 alone (in doses of 10 mg daily of pyridoxine resulting in a 5% homocysteine reduction) appears smaller than that of folic acid alone (5 mg daily causing 45 to 50% decrease) [20]. Several studies have shown reductions in homocysteine levels of 30 to 50% with various B-complex multivitamin regimens, whereas others have shown similar decreases with folic acid alone. However, most of these trials concerned hyperhomocysteinemic patients not on dialysis. Since hemodialysis patients are not only hyperhomocysteinemic as a group, but also at risk for deficiencies in hydrosoluble vitamins, it seems even more appropriate to supplement them aggressively.

A mutation (an alanine-to-valine substitution, T to C) in MTHFR renders it thermolabile and may be associated with elevated levels of homocysteine [45]. The incidence of the MTHFR mutation genotypes in our hemodialysis population is very similar to the reported incidence in the general population [45]. We observed a slightly different degree of reduction of serum homocysteine for the different MTHFR genotypes. Homozygotes (TT) presented the highest tHcy concentrations at baseline, as well as the greater reduction in tHcy levels as a group (41.2%), but nevertheless ended up after 12 months with final tHcy values similar to the patients with either normal (CC) or heterozygous (CT) genotypes. Many nonrenal-failure individuals homozygous for the MTHFR mutation have normal serum homocysteine levels; it has been proposed that the homozygous genotype is not necessarily associated with hyperhomocysteinemia except if a concomitant deficiency in folate occurs [43]. Thus, individuals homozygous for the mutation may have higher folate requirements for the regulation of plasma homocysteine. Interestingly, other investigators have shown that the folate status and the MTHFR mutation interact at a much higher folate level in dialysis patients (folate 29.2 ng/mL) when compared with a population with normal renal function (folate 6.8 ng/mL) [48]. Even though our patients had a normal folate status at the beginning of the study, those with the homozygous genotype had higher homocysteine values and appeared more responsive to additional intravenous folic acid after six months.

To summarize, (*1*) hyperhomocysteinemia was present in 100% of our 168 chronic hemodialysis patients. (*2*) The distribution of their MTHFR genotypes appeared comparable to the one prevailing in the French Canadian population. (*3*) Considering the entire unsupplemented group at baseline, nearly 40% of our patients were deficient in vitamin B_6. Few of them presented low serum concentrations of vitamin B_{12}, and most of them had a normal folate status. (*4*) Supplementation with DiaVite® (with or without added intravenous folic acid) has resulted in very significant tHcy reductions in serum tHcy for all three genotypes (by more than 29% at 6 months and by close to 35% at 12 months). (*5*) The impact of the proposed supplementation protocol was found after 6 months and was maintained but did not increase further

after 12 months of the same regimen. (*6*) The addition of intravenous folic acid has been associated with a more pronounced decrease at six months, particularly in TT patients. However, further benefits were not clearly observed thereafter.

The present study was not designed to assess the effects of supplementation with folic acid, vitamin B_6, or vitamin B_{12} separately; therefore, the potential impact of each compound cannot really be determined. However, our results, obtained in a large cohort of chronic hemodialysis patients followed over one year, suggest that a hydrosoluble multivitamin enteral formulation, such as DiaVite®, is desirable in ESRD patients treated by high-efficiency hemodialysis to normalize serum concentrations of B-complex vitamins. Its administration leads to very significant reductions in serum tHcy, which over the long-term may prove to be beneficial as a preventive measure. Supraphysiologic doses of folic acid may be especially useful to lower hyperhomocysteinemia in hemodialysis patients homozygotes for the TT genotype in the initial months of DiaVite® treatment.

ACKNOWLEDGMENTS

Dr. Leblanc is supported by Fonds de la Recherche en Santé du Québec (FRSQ).

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