



Efficacy and safety of two vitamin supplements regimens on homocysteine levels in dialysis patients. Prospective and randomized clinical trial

J. E. Sánchez Álvarez, L. Pérez Tamajón*, D. Hernández*, A. Álvarez González, P. Delgado and V. Lorenzo*

Hospital Tamaragua. *Hospital Universitario de Canarias. Tenerife.

SUMMARY

High levels of homocysteine (tHcy) are frequent in MHD patients, and recognized as a risk factor for cardiovascular events. Vitamin supplements have been shown to lower serum Hcys, although optimal dose and efficacy is not well defined. Moreover, methylenetetrahydrofolate reductase (MTHFR) gene polymorphism can modulate its prevalence and response to treatment. Objective: To evaluate efficacy and safety of two vitamin supplement regimens on Hcys serum levels over a 12 month period.

Methods: We conducted a prospective, randomised, double-blind trial in 60 stable MHD patients (68 ± 13 years, 48% male, 50% diabetics). Patients were randomly assigned to one of two treatment regimens: 1) daily renal multivitamin containing folate (FA), vitamin B₆ and B₁₂ (5 mg, 10 mg and 0.4 mg respectively) (N = 27); and 2) supraphysiological daily doses (15 mg, 100 mg and 1 mg) (N = 33). These regimens were continued throughout the study period. Hcys levels were compared with a control group from the general population (N = 276) matched for age and gender. Measurements: demographic and clinical data, serum levels of Hcys, FA, B₆, B₁₂ at baseline and after 1, 3, 6 and 12 months of treatment; MTHFR gene polymorphism (PCRRT).

Results: At baseline, global prevalence of hyperhomocysteinemia (tHcy ≥ 15 pmol/L) was 100% in patients and 22% in controls. Hcys levels were significantly higher in patients versus controls (32.4 ± 8.9 vs 12.9 ± 6.8 ; $P < 0.0001$). Both regimens were equally effective in reducing Hcys levels. As a whole, Hcys levels were reduced by 23.6% ($P < 0.001$) after one month of treatment. The highest reduction was observed at the sixth month (28.3%, 32.4 ± 8.9 vs 22.7 ± 6.4 , $P < 0.001$) and remained stable thereafter. However, only 12% of patients normalised their plasma levels after 12 months of therapy. The effect of treatment was not influenced by age, gender, diabetes, body weight or time on MHD. Reduction rate of tHcy levels was related to baseline tHcy level ($r = 0.500$, $P < 0.001$) and baseline FA levels ($r = -0.332$, $P = 0.009$). The MTHFR polymorphism did not significantly modify the effect of the treatment. No side effects were associated with either regimen.

Recibido: 10-V-2004

En versión definitiva: 23-IX-2004

Aceptado: 25-IX-2004.

Correspondence: Dr. Víctor Lorenzo Sellarés
Servicio de Nefrología
Hospital Universitario de Canarias
Carretera La Cuesta-Taco. La Laguna, Tenerife.
E-mail: lorenzovictor@terra.es

Conclusions: *Hyperhomocysteinemia is common in patients with conventional HD schedules and this is not related to vitamin deficiencies. Vitamin supplements significantly reduce Hcys levels in a sustained but suboptimal way. Supraphysiological doses did not improve the results. Further studies are required to demonstrate that this effect is associated with an improval in morbidity and mortality.*

Key words: **Homocysteine. Folate. Vitamin B₁₂. Vitamin B₆. Hemodialysis. Atherosclerosis.**

EFICACIA Y SEGURIDAD DE DOS PAUTAS DE SUPLEMENTOS VITAMÍNICOS SOBRE LOS NIVELES DE HOMOCISTEÍNA EN PACIENTES EN HEMODIÁLISIS. ENSAYO CLÍNICO PROSPECTIVO Y RANDOMIZADO

RESUMEN

La hiperhomocisteinemia (HHcys) es frecuente en pacientes urémicos y representa un factor de riesgo cardiovascular. Los suplementos vitamínicos reducen los niveles de homocisteína (Hcys), no estando bien definida la dosis óptima y su eficacia. El genotipo de la metilentetrahidrofolato reductasa (MTHFR) puede modular su prevalencia y respuesta al tratamiento.

Objetivo: *conocer la eficacia de dos pautas de suplementos vitamínicos sobre los niveles de Hcys tras 12 meses de tratamiento.*

Metodología: *estudio prospectivo, randomizado, a doble ciego. Sesenta pacientes en hemodiálisis se aleatorizaron en dos grupos terapéuticos: A) Dosis habituales de ácido fólico (AcF), vitamina B₆ y B₁₂ a la dosis de 5, 10 y 0.4 mg diarios, respectivamente (N = 27) y B) Dosis suprafisiológicas (15, 100 y 1 mg diarios) (N = 33). Los valores de Hcys se compararon con un grupo control similar en edad y sexo.*

Resultados: *Todos los pacientes y el 22% de los controles presentaron HHcys siendo los niveles de Hcys 2,5 veces superiores en los pacientes que en el grupo control ($32,4 \pm 8,9$ vs $12,9 \pm 6,8$ $\mu\text{mol/L}$; $P < 0,0001$). Las dos pautas fueron igualmente eficaces en reducir la Hcys, aunque solamente el 12% de los pacientes normalizaron sus niveles al finalizar el estudio. Globalmente, los niveles de Hcys experimentaron un descenso significativo al mes de tratamiento (23,6%, $P < 0,001$). El mayor descenso se registró tras 6 meses (28,3%, $P < 0,001$), estabilizándose hasta los 12 meses. El mayor descenso de Hcys se asoció con niveles basales más altos de Hcys ($r = 0,500$, $P < 0,001$) y más bajos de AcF ($r = 0,332$, $P = 0,009$). El polimorfismo de la MTHFR no modificó significativamente la respuesta terapéutica.*

Conclusiones: *La HHcys es constante con pautas convencionales de hemodiálisis aunque no existan déficits vitamínicos. Los suplementos reducen de forma substancial y sostenida los niveles de Hcys. Dosis suprafisiológicas no suponen ventajas adicionales. Se requieren nuevos estudios para demostrar que este descenso conlleve un impacto final favorable sobre la morbimortalidad cardiovascular.*

Palabras clave: **Homocisteína. Folato. Vitamina B₁₂. Vitamina B₆. Hemodiálisis. Aterosclerosis.**

INTRODUCCIÓN

The existence of a more aged, atheromatous and diabetic population on dialysis justifies the increasing interest and concern that represent cardiovascular (CV) events in this population. CV complications account for 45-50% of all death causes in dialysis¹, and mortality from CV origin is clearly higher in the uremic population than in the normal population.² In fact, renal insufficient patients have the same cardiovascular risk factors than those in the general population^{3,4}, in addition to those related with the uremic status itself, such as secondary hyperparathyroidism, anemia, and uremia itself.⁵

Hyperhomocysteinemia (HHcys) is risk factor for arteriosclerosis and veno-occlusive disease in the general population⁶ and in renal failure.⁷ HHcys is a universal phenomenon in the uremic population, homocysteine (Hcys) levels being 2-3 times higher than those of an age- and gender-matched general population.⁸ Its origin is multifactorial and includes genetic predisposition,⁹ nutritional deficit of cofactors for Hcys metabolism,^{10,11,12} and renal failure itself.¹³ It seems that uremic status by itself influences on the increase of Hcys levels. If that were the case, it would be foreseeable that a higher dialysis dose will have a favorable impact on Hcys levels, as a recent work seems to confirm.¹⁴

It has been demonstrated that vitamins B₆, B₁₂ and folic acid (FAC) effectively reduce Hcys levels in the general population.^{15,16} However, in the uremic population vitamin therapy has yielded poorer results.^{17,18,19,20} These studies have been done with relatively short follow-up periods, with low number of patients, and optimal dose has not been established. Besides, there is controversy whether Hcys levels decrease represents a benefit²¹ or not²² on patient's survival.

We set up a prospective, randomized clinical trial to know the effect of two vitamin supplement regimens on Hcys levels after a 12-months treatment in a population on hemodialysis. Moreover, we studied HHcys prevalence and its relationship with vitamins B₆, B₁₂ and FAC serum levels. Additionally, we assessed the influence of methyltetrahydrofolate reductase (MTHFR) enzyme polymorphism on Hcys levels and on response to treatment.

MATERIAL AND METHODS

Patients

All patients on hemodialysis (HD) program from Tamaragua Hospital (Puerto de la Cruz, Tenerife), to date April/2002, were assessed for the present study.

Dialysis regimen was 12-13.5 hours/week, three times a week, with a prescribed percentage of urea reduction \leq 65%, using a polysulphone F8 dialyzer (Fresenius. Bad. Homburg., Germany).

Inclusion criteria were: 1) patients of both genders, older than 18 years, clinically stable defined as absence of complications that have required hospital admission within the last 3 months, with exception of those related to the vascular access; 2) being on HD program for longer than 6 months; 3) Negative pregnancy test in women; 4) Absence of known or eventual intolerance reaction to vitamin complex.

Biochemical measurements

Blood samples were drawn at the beginning of HD session, at the middle of the week. The general analysis was performed with an autoanalyzer by means of routine techniques. FAC and vitamin B₁₂ levels were simultaneously determined by a radiometric method (SPNB, DualCount Folic Acid/Vitamin B₁₂ in-vitro Diagnostic Test Kit; Diagnostic Products Corporation; Los Angeles; California); Normal FAC reference values for the study were 3.0-17.0 nmol/L and for Vitamin B₁₂ 200-950 pmol/L. Vitamin B₆ was determined by radioenzimatic method (reference values 20-160 nmol/L).

Total homocysteine levels (the sum of free form and the one bound to proteins) were determined by using a fluorescent polarization immunoassay (IMX, Abbot Diagnostics, Abbot Park, IL.)

MTHFR genotype

Patients' DNAs were submitted to two allele-specific amplifications by polymerase chain reaction (PCR), using a common nucleotide and a specific one, either for Ala223 allele or to Val223 allele. Primers design was done from the sequence deposited at GenBank (U09806).

Statistical analysis

Continuous variables were expressed as means and standard deviation, and categorical variables as percentage. Baseline values for both therapeutic groups were compared by t test and Chi-squared test, as indicated. Previously, Kolmogorov-Smirnov test was used to check for a normal distribution. In order to analyze Hcys levels during the study period, a general linear model with repeated measurements was used. Percentage and absolute changes in Hcys were compared between both treatment groups by t test or

Mann-Whitney U test, as indicated. For univariate correlations, Pearson's *r* was used. In order to know the best baseline Hcys predictors and in response to treatment a linear regression model was applied. SPSS statistical software (11.1® for Windows) was used (SPSS Inc., Chicago, IL) for outcome analysis.

RESULTS

Baseline parameters

Finally, 60 patients were included. Vital statistics and baseline biochemical data for all patients and for both treatment groups are shown in table I.

Baseline values for Hcys, FAc, and vitamin B₁₂ for the whole group of patients and the control group are compared in Figure 1. Mean Hcys baseline values were 2.5 fold higher in patients than controls.

HHcys was defined when Hcys levels were higher than 15 µmol/L.²³ With this cut-off point, in Figure 1A we may observe that 100% of patients and 22% of controls had HHcys, respectively. In Figures 1B and 1C is shown that baseline FAc and vitamin B₁₂ values, respectively, were significantly higher in patients than controls.

Predictors for baseline Hcys levels

In univariate analysis, baseline Hcys levels were only negatively correlated to baseline FAc values (*r* = 0.340; *P* = 0.008). We could not find an association between baseline Hcys levels with other biochemical parameters (vitamins B₆ and B₁₂ levels, lipid profile, glucose, BUN, albumin) or vital statistics (age, gender, time on HD, diabetes, or AHT).

In order to know the best predictors of Hcys baseline levels, we used linear regression model including demographic, biochemical, and MTHFR genotype parameters as independent variables. With this model, only FAc baseline levels were independent predictors of Hcys (*B* = -0.75 [95%CI = -1.21, -0.31], *p* < 0.001).

Response to treatment

Of the 60 initial patients, 54 completed the study, three were transplanted and three died. Treatment was well tolerated, without any adverse effect or medication withdrawal for that reason. Progression of FAc, and vitamins B₆ and B₁₂ during treatment period of both groups is shown in figure 2. These data confirmed the convenient patients' adherence to treatment observed

Table I. Vital statistics and biochemical values for both treatment groups. Both demographic and levels for Hcys and the three vitamins were similar in both groups

	Total	Group 1	Group 2
<i>N</i>	60	30	30
<i>Age (years)</i>	68 ± 13	64 ± 14	70 ± 10
<i>Males %</i>	48	48	48
<i>Diabetics %</i>	50	50	50
<i>AHT %</i>	13	14	12
<i>Hcys (µmol/L)</i>	32.4 ± 8.9	32.7 ± 7.9	32.1 ± 9.8
<i>FAc (nmol/L)</i>	7.74 ± 7.3	8.0 ± 9.6	7.5 ± 4.8
<i>B₁₂ (pmol/L)</i>	546.6 ± 222	516.8 ± 185	570.9 ± 248
<i>B₆ (nmol/L)</i>	32.9 ± 49.4	31.1 ± 47.7	34.4 ± 51.5

during when supervising the collection of medication blisters. FAc and vitamins levels significantly increased in each group, especially during the first trimester. Although both groups received different dosage, the level differences between both groups were not relevant, except for FAc at month 6 of treatment (14.74 ± 8.20 vs 26.48 ± 28.22 mmol/L, *p* < 0.003).

Globally, Hcys levels decreased by 24% during the first trimester. The greatest decrease was observed at month 6 (28.3%), remaining stable throughout the follow-up period. The effect of both treatment regimens on Hcys levels is shown in figure 3. With the use of usual doses, Hcys levels decreased by 20%, 26%, 30%, and 33% at treatment months 1, 3, 6, and 12, respectively. A similar decrease by 22%, 28%, 27% and 24% was observed in patients that received supraphysiologic doses.

Although the use of vitamin supplement was effective in decreasing Hcys levels, this decrease was suboptimal since only 12% of patients normalized Hcys values. Changes in Hcys levels before and after a 12-months treatment are shown in figures 4.

Predictors of response to treatment

In the univariate analysis, we observed that the delta (Δ) for Hcys decrease (percentage of decrease from baseline) after 12 months of treatment was directly associated with Hcys baseline levels (*r* = 0.5, *P* < 0.001) and negatively correlated to FAc baseline levels (*r* = -0.332, *P* = 0.009) (figures 5A and 5B). Other demographic (age, gender, diabetes, time on HD) or biochemical (baseline vitamins

B₆ and B₁₂ levels, lipid profile, parameters, glucose, albumin, or BUN levels) did not have an effect. With the multivariate linear regression model only baseline Hcys levels were predictive of response to treatment (B = 0.97 95%CI = 0.39, 1.54], P < 0.001).

Effect of MTHFR gene polymorphism

Our patients were grouped in the following way according to MTHFR gene polymorphism: 7 (12%) were carriers of an unfavorable genotype (VV); the remaining 24 patients (40%) carried the AA genotype, and 29 (48%) the AV genotype. These proportions are similar to those described in the general population^{17,21}.

Regarding its influence, we could observe that there were no significant differences between Hcys and FAc levels, or Hcys delta between patients with an unfavorable genotype and those with favorable genotype.

DISCUSSION

This work shows that HHcys is virtually universal in patients on HD, even without a vitamin deficiency. The clinical study results demonstrate that a safe and cheap, with two different doses, water-soluble vitamin complex (FAc, vitamins B₆ and B₁₂) is effective in reducing Hcys levels, although only in a small percentage it reaches the normality range.

Criteria used to define HHcys are not uniform in the literature.^{6,17,18,24,25} This is due to the particular

characteristics and design for each study. Some authors have defined it as the value above which the number of CV events or atherosclerotic or arteriosclerotic damage of the vascular tree is significantly increased.²⁶ Others have applied the extreme ranges of their series, in terms of percentiles or standard deviations.^{27,28} In spite of the discrepancies, most of the authors use 12-16 μmol/L values as the cut-off point above which HHcys is defined. When applying 15 μmol/L as the cut-off point,²³ we agree with other authors in that virtually 100% of patients on HD present HHcys.

Serum vitamin levels are highly variable, both in the general^{24,29,30} and uremic populations.^{7,31,32} In dialysis patients high,¹⁸ normal,³¹ and low¹⁹ values have been described. Of note, several authors have pointed out that HHcys occurs in HD patients in spite of normal or above normal vitamins levels.^{7,17,33} In fact, in our control population vitamins levels were lower than in patients, although the mean and median for these values were within the normal range in both populations. These data suggest that it is not vitamin deficiency or losses due to dialysis what conditions HHcys in dialysis patients and that other factors related to uremia itself may be involved.

Bostom et al.¹⁷ showed the beneficial effect of supraphysiologic doses of vitamins (FAc, 16 mg/day) as compared to the control group (FAc, 1 mg/day), reaching a decrease of Hcys values by 30% vs. 2%, respectively, after 8 weeks of treatment. We used different vitamin complex doses, achieving a similar effect in both cases and identical to that achieved by Boston with supraphysiologic doses with his complex. Other authors using a combination of vitamin complexes and regi-

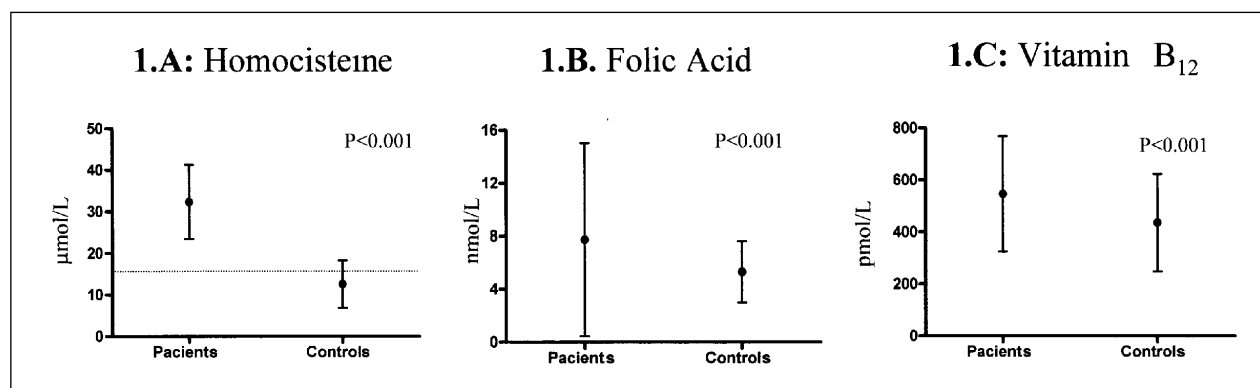


Fig. 1.—In figures 1A, 1B and 1C values (mean ± SD) for Hcys, FAc, and B₁₂ are respectively compared between patients and the control group. In Figure 1A, the dotted line represents the 15 μmol/L value taken as the reference threshold.

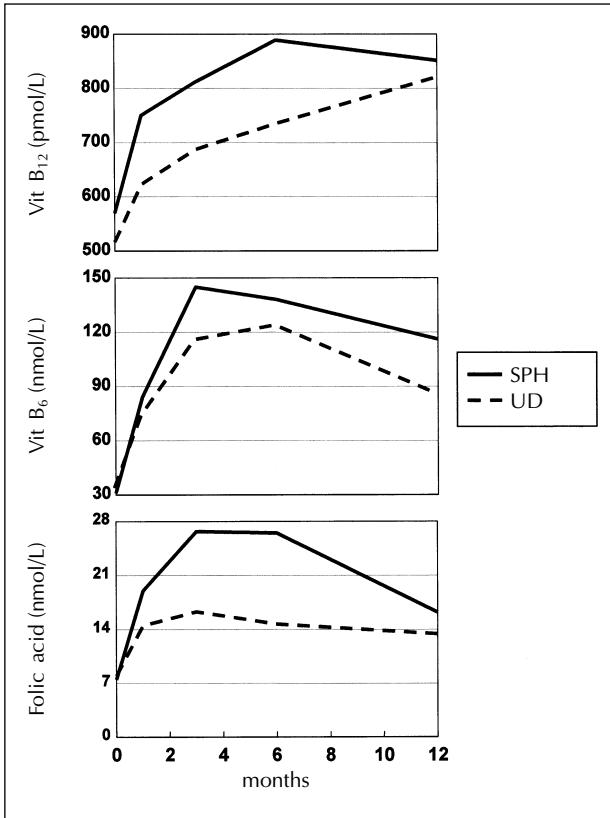


Fig. 2.—FAC, B12, and B6 during the treatment period in both treatment groups (SPH: supraphysiologic doses; UD: usual doses)

mens^{17,25,32,33,34} have shown similar results, i.e., Hcys levels decrease around 20-35%, but only a small proportion reaches normal values. Generally, treatment periods in these series have not exceeded 6 months. Tremblay et al.¹⁸ studied the effect of vitamin supplements in 168 HD patients for 12 months. All patients received a multivitamin complex daily (FAC 1 mg, B₆ 10 mg, B₁₂ 6 µg). Besides, half of them received FAC 10 mg i.v. postdialysis. They observed a mean decrease in Hcys levels of 23.5% at 6 months, and of 21.7% at one year. The group with extra FAC doses postdialysis showed a significant decrease, greater than the one of the other group at 6 months, but without differences at one year.

Our study was designed in the long term, 12 months, observing that once a maximum decrease level has been reached —by the 6th month— Hcys levels remain stable. We must point out the stabilization of Hcys decrease, without normalizing. Several factors have been proposed to explain this circumstance, including the loss of renal metabolism,¹³

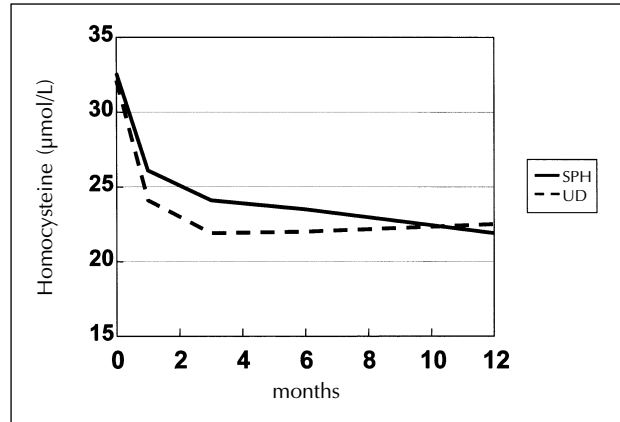


Fig. 3.—Hcys levels during the treatment period in both treatment groups (SPH: supraphysiologic doses; UD: usual doses).

and the decrease of glomerular filtration.³⁵ This could explain the increase of Hcys in renal failure but not the exhaustion of vitamins effect.

Other observations to consider in this study are: 1) there is an inverse relationship between Hcys and FAC baseline levels, but not with vitamin B; 2) lower FAC levels and higher Hcys levels are independent predictors of treatment efficacy; and 3) high Hcys levels were present even in those patients in the higher fourth of folic acid blood levels (FAC > 8.7 mmol/L). All these data suggest that the effect of FAC on Hcys levels is quantitatively more relevant than the one of vitamin B, and that factors related to uremic status itself favor a resistance to vitamins effect.

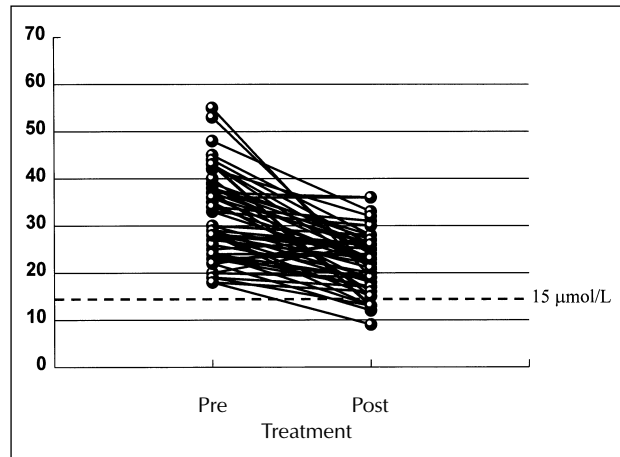


Fig. 4.—Individual Hcys baseline levels and at 12 months of treatment. The dotted line represents Hcys value at 15 µmol/L.

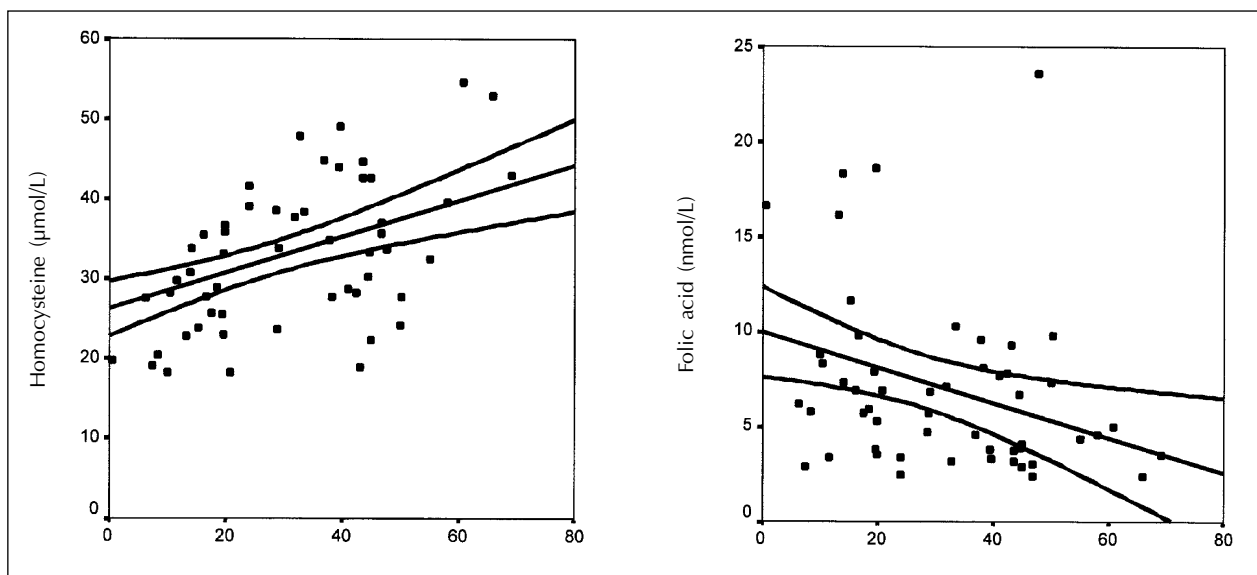


Fig. 5.—Correlation between delta decrease of homocysteine and baseline homocysteine levels (Figure 5A) and folic acid levels (Figure 5B).

The present study demonstrates that a vitamin complex at the used doses effectively and steadily decreases Hcys levels. However, only 12% of patients normalized Hcys levels. These apparently suboptimal results should not be considered ineffective. It has been observed that mortality is associated to a proportional increase in Hcys levels in coronary heart disease patients.³⁶ A large meta-analysis of studies performed in the general population has shown that for each 3 µmol/L decrease in Hcys the risk for coronary heart disease, deep venous thrombosis, and cerebrovascular attack is decreased by 16%, 25%, and 24%, respectively.³⁷ Assuming that the same may happen in HD patients, any decrease achieved in Hcys levels ought to have a favorable impact on CV mortality and morbidity in this population. Thus, considering the alarming magnitude of CV complications in HD, it seems reasonable to suggest routine use of these safe and cheap vitamin supplements, at standard doses, in these patients. Because of the universality of HHcys in HD, the use of these vitamins is an issue to consider, even without the availability of routine serum Hcys determinations.

Regarding MTHFR gene influence, our results are clearly limited by sample size (the unfavorable genotype is present in only 7 patients). It is well known that large samples are required to identify any association between polymorphisms and clinical observations or therapeutic outcomes. With the data ob-

served by us, we cannot affirm that MTHFR polymorphism has any influence on baseline Hcys levels or on treatment response.

To conclude, HHcys is constant in patients with conventional HD regimens, although not having vitamin deficiencies. However, the present clinical trial shows a beneficial effect, although suboptimal, of vitamin supplements at different doses on Hcys levels. From these observations, we may deduce that uremic status itself favors a partial resistance to the effect of vitamins. If this were to be true, it would be expected that a higher dialysis dose will have a favorable impact on Hcys levels. It is an essential issue, pending to demonstration, whether the beneficial effect of these treatment modalities on Hcys levels leads to a final favorable impact on cardiovascular mortality and morbidity.

REFERENCES

1. U.S. Renal Data System; USRDT 2002. Annual Data Report: National Institute of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2002.
2. Foley RN, Parfrey PS, Sarnak MJ: Clinical epidemiology of cardiovascular disease in chronic renal disease. *Am J Kidney Dis* 32: 5112-5119, 1998.
3. Ma K, Greene E, Raij L: Cardiovascular risk factors in chronic renal failure and hemodialysis populations. *Am J Kidney Dis* 21: 505-513, 1992.

4. Rostand S, Brunzell J, Cannon R, Victor R: Cardiovascular complications in renal failure. *J Am Soc Nephrol* 2: 1053-1062, 1991.
5. JC Ayus, V Lorenzo, D Hernández: Complicaciones cardíacas en la insuficiencia renal crónica y después del trasplante renal. En: Llach F, Valderrábano F (eds.): *Insuficiencia Renal Crónica. Diálisis y Trasplante Renal*. Ediciones Norma, Madrid, pp. 173-198, 1997.
6. Boushey CJ, Beresford SA, 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* 274: 1049-1057, 1995.
7. Manns B, Burgess E, Hyndman ME, Parsons HG, Schaefer JP, Scott-Douglas NW: Hyperhomocysteinemia and the prevalence of atherosclerotic vascular disease in patients with end-stage renal disease. *Am J Kidney Dis* 34: 669-677, 1999.
8. Bostom A, Lathrop L: Hyperhomocysteinemia in end-stage renal disease: Prevalence, etiology, and potential relationship to arteriosclerotic outcomes. *Kidney Int* 52: 10-20, 1997.
9. Fodinger M, Mannhalter C, Wolf G, Pabinger I, Muller E, Schmid R, Horl WH, Sunder-Plassmann G: Mutation (677 C to T) in the methylenetetrahydrofolate reductase gene aggravates hyperhomocysteinemia in hemodialysis patients. *Kidney Int* 52(2): 517-523, 1997.
10. Bachmann J, Tepel M, Raidt H, Riezler R, Graefe U, Langer K y cols.: Hyperhomocysteinemia and the risk for vascular disease in hemodialysis patients. *J Am Soc Nephrol* 6(1): 121-125, 1995.
11. Bostom AG, Shemin D, Lapane KL, Nadeau MR, Sutherland P, Chan J, Rozen R, Yoburn D, Jacques PF, Selhub J, Rosenberg IH: Folate status is the major determinant of fasting total plasma homocysteine levels in maintenance dialysis patients. *Atherosclerosis* 123: 193-202, 1996.
12. Van Guldener C, Janssen MJ, de Meer K, Donker AJ, Stehouwer CD: Effect of folic acid and betaine on fasting and postmethionine-loading plasma homocysteine and methionine levels in chronic haemodialysis patients. *J Intern Med* 245(2): 175-83, 1999.
13. Van Guldener C, Stam F, Stehouwer CDA: Homocysteine metabolism in renal failure. *Kid Intern* 59 (suppl 78): 234-237, 2001.
14. Maduell F, Navarro V, Torregrosa E, Rius A, Dicenta F, Cruz MC, Ferrero JA: Change from three times a week on-line hemodiafiltration to short daily on-line hemodiafiltration. *Kidney Int* 64: 305-13, 2003.
15. Ubbink J, Hayward Vermaak WJ, Merwe A, Becker PJ, Delport R, Potgieter HC: Vitamin requirements for the treatment of hyperhomocysteinemia in humans. *J Nutr* 124: 1927-1933, 1994.
16. Clarke R on behalf of Homocysteine Lowering Trialists' Collaboration: Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. *BMJ* 316: 894-8, 1998.
17. Bostom AG, Shemin D, Lapane KL, Hume AL, Yoburn D, Nadeau MR, Bendich A, Selhub J, Rosenberg IH: High Dose B-Vitamin treatment of hyperhomocysteinemia in dialysis patients. *Kidney Int* 49: 147-152, 1996.
18. Tremblay R, Bonnardeaux A, Geadah D, Busque L, Lebrun M, Ouimet D, Leblanc M: Hyperhomocysteinemia in hemodialysis patients: effects of 12-month supplementation with hydrosoluble vitamins. *Kidney Int* 58(2): 851-8, 2000.
19. Ducloux D, Aboubakr, Motte G, Toubin G, Fournier V, Chalopin JM, Drüeke T, Massy A: Hyperhomocysteinemia therapy in hemodialysis patients: folinic versus folic acid in combination with vitamin B₆ and B₁₂. *Nephrol Dial Transplant* 17: 865-870, 2002.
20. Mamzs B, Hyndman E, Burgess E, parson H, Schaefer J, Snyder F, Scott-Douglas N: Oral vitamin B₁₂ and high-dose folic acid in hemodialysis patients with hyper-homocysteinemia. *Kidney Int* 59: 1103-1109, 2001.
21. Schnyder G, Roffi M, Pin R, Flammer Y, Lange H, Eberu FR, Meier B, Turi ZG, Hess OH: Decreased rate of coronary restenosis after lowering of plasma homocysteine levels. *N Engl J Med* 345: 1593-1600, 2001.
22. Wrone EM, Homberger JM, Zehnder JL, McCann LM, Coplion NS, Fortmann SP: J Randomized trial of folic acid for prevention of cardiovascular events in end-stage renal disease. *J Am Soc Nephrol* 15: 420-426, 2004.
23. Refsum H, Smith AD, Ueland PM, Nexø E, Clarke R, McPartlin J, Johnston C, Engbaek F, Schneede J, McPartlin C, Scott JM: Facts and recommendations about total homocysteine determinations: an expert opinion. *Clin Chem* 50: 3-32, 2004.
24. Graham IM, Daly LE, Refsum HM, Robinson K, Brattström LE, Ueland PM y cols.: Plasma homocysteine as a risk factor for vascular disease: the European Concerted Action Project. *JAMA* 277: 1774-1781, 1997.
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* 51(2): 108-15, 1999.
26. Moustapha A, Naso A, Nahlawi M, Gupta A, Arheart KL, Jacobsen DW, Robinson K, Dennis VW: Prospective study of hyperhomocysteinemia as an adverse cardiovascular risk factor in ESRD. *Circulation* 97: 138-141, 1998.
27. Moustapha A, Gupta A, Robinson K, Arheart K, Jacobsen DW, Schreiber MJ, Dennis VW: Prevalence and determinants of hyperhomocysteinemia in hemodialysis and peritoneal dialysis. *Kidney Int* 55(4): 1470-5, 1999.
28. Perna A, Ingrassio D, de Santo N, Galletti P, Brunone M, Zappia V: Metabolic consequences of folate-induced reduction of hyperhomocysteinemia in uremia. *J Am Soc Nephrol* 8: 1899-1905, 1997.
29. Ubbink JB, Vermaak WJH, Van de Merwe A, Becker PJ: Vitamin B₁₂, vitamin B₆, and folate nutritional status in men with hyperhomocysteinemia. *Am J Clin Nutr* 57(1): 47-53, 1993.
30. Robinson K, Mayer EL, Miller DP, Green R, van Lente F, Gupta A, Kottke-Marchant K, Savon SR, Selhub J, Nissen SE, Kutner M, Topol EJ, Jacobsen DW: Hyperhomocysteinemia and low pyridoxal phosphate. *Circulation* 92: 2825-2830, 1995.
31. Armada E, Pérez Melón C, Otero A, Gayoso P, Rodríguez M, Esteban J: Efecto de la suplementación con ácido fólico sobre los niveles de homocisteína total en pacientes en hemodiálisis. *Neurología* 21: 1671-73, 2001.
32. Bostom AG, Shemin D, Gohh RY, Beaulieu J, Bagley P, Massy ZA, Jacques PF, Dworkin L, Selhub J: Treatment of hyperhomocysteinemia in hemodialysis patients and renal transplant recipients. *Kid Intern* 59 (suppl 178): 246-252, 2001.
33. Sunder-Plassmann G, Fodinger M, Buchmayer H, Papiannopoulos M, Wojcik J, Kletzmayer J, Enzenberger B, Janata O, Winkelmayer WC, Paul G, Auinger M, Barras U, Horl WH: Effect of high dose folic acid therapy on hyperhomocysteinemia in hemodialysis patients: results of the Vienna multicenter study. *J Am Soc Nephrol* 11(6): 1106-16, 2000.
34. Amadottir M, Brattstrom M, Simonsen O, Thysel H, Hultberg B, Andersson A: The effect of highdose pyridoxine and folic acid supplementation on serum lipid and plasma homocysteine concentration in dialysis patients. *Clin Nephrol* 40: 236-240, 1993.

J. E. SÁNCHEZ ÁLVAREZ y cols.

35. Clarke R, Lewington S, Landray M: Homocysteine, renal function and risk of cardiovascular disease. *Kid Intern* 63 (suppl 84): 131-133, 2003.
36. Nygard O, Nordrehaug J, Refsum H, Ueland P, Farstad M, Farstad M, Vollset S: Plasma homocysteine levels and mortality in patients with coronary artery disease. *N Eng J Med* 337: 230-6, 1997.
37. Wald DS, Law M, Morris JK: Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. *BMJ*, vol. 325, 23 nov 2002.