Hyperhomocysteinemia and Related Factors in 600 Hospitalized Elderly Subjects

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Hyperhomocysteinemia (HHcy) is a metabolic disorder frequently occurring in the elderly population. Recently several reports have suggested abnormalities in homocysteine (tHcy) metabolism implicating HHcy as a metabolic link in the multifactorial processes characterizing many geriatric illnesses—with special emphasis on atherosclerotic vascular diseases and cognitive impairment. The present study was undertaken in a large sample of elderly hospitalized subjects to determine (1) the prevalence of HHcy, (2) the association of HHcy with vascular and cognitive disorders, and (3) the factors independently predicting Hhcy. Six hundred elderly subjects (264 men and 336 women; mean age, 79 ± 9 years) were randomly chosen from those admitted as inpatients over a period of 3 years. In all patients, body mass index (BMI), mid-upper arm muscle area (MUAMA), plasma cholesterol, triglycerides, total proteins, albumin, lymphocyte count, creatinine, homocysteine (fasting and 4 hours after methionine oral load), serum vitamin B_6, vitamin B_{12}, and folate concentrations were measured. The presence of disease or use of medications known to affect homocysteine plasma levels were also recorded. The mean fasting tHcy level was 16.8 ± 12 μmol/L in the whole sample, 18.18 ± 13.25 μmol/L in men, and 15.86 ± 12.14 μmol/L in women (*P* = .005 men *v* women). The mean Hcy level 4 hours after methionine load was 37.95 ± 20.9 in the whole sample. Prevalence of hyperhomocysteinemia (fasting Hcy ≥ 15 μmol/L or 4 hours after methionine load ≥ 35 μmol/L) was 61% (365/600) (67% in men and 56% in women, *P* < .05). HHcy was rarely (8%) an isolated disorder; in addition to diabetes (20%), renal failure (48.2%), and malnutrition (20.2%), it was often associated with heart failure (30%), malignancies (20.5%), and the use of diuretics (56%) and anticonvulsant drugs (13%). Plasma homocysteine progressively increases across subjects from those with no diabetes, malnutrition, renal failure, obesity, inflammatory bowel disease, heart failure to those with 1, 2, or more concurrent diseases. Multiple stepwise regression analysis showed that 72% of plasma total fasting tHcy variability was explained by age, serum folate, plasma albumin, use of diuretics, and renal function (measured as plasma creatinine clearance). In conclusion, the present study documents that hyperhomocysteinemia, in elderly hospitalized patients is (1) a common finding, (2) frequently associated with vascular and cognitive disorders, and (3) probably a secondary phenomenon in most cases. The major predictor of high plasma homocysteine levels were age, serum folate, plasma albumin, plasma creatinine clearance, and use of diuretic drugs. These variables explain a large proportion of plasma Hcy variability.

HYPERHOMOCYSTEINEMIA (HHcy) is a metabolic disorder that occurs frequently in the elderly population.[1-4] Elevated total homocysteine (tHcy) levels at fasting or after oral methionine load have been associated with a higher risk of vascular disease (atherosclerosis and venous thromboembolism).[5-7] Recently, HHcy in elderly subjects has also been associated with cognitive impairment.[8-11] Accordingly, some investigators have suggested disorders in homocysteine metabolism leading to HHcy as a metabolic link in the multifactorial processes characterizing many geriatric illnesses, with special emphasis on atherosclerotic vascular diseases and dementia.[12,13]

The overall prevalence of HHcy in the healthy elderly is about 30% to 40%, and it may be even greater in the general elderly population (54% to 56%).[1,14] This prevalence is expected to be much higher in hospitalized patients, but few studies have focused on this issue.[1]

Although it is not easy to distinguish between primary (genetic) and secondary (acquired forms) HHcy, in the majority of elderly subjects, it seems to be a secondary phenomenon sustained by underlying disease (renal disorders) or exogenous factors, such as medications or vitamin deficits, which increase the production and/or influence the renal handling of homocysteine. In a minority of persons, HHcy may be thought to be a primary abnormality.[15-19]

The relative role exerted by different factors influencing plasma tHcy levels in the elderly hospitalized population has been poorly assessed. Moreover, the number of studies formally exploring the association of HHcy with vascular disease and cognitive impairment in the elderly is insufficient.

The present study was undertaken in a large sample of elderly hospitalised subjects to determine (1) the prevalence of hyperhomocysteinemia, (2) the association of HHcy with vascular and cognitive disorders, and (3) factors independently predicting HHcy.

MATERIALS AND METHODS

Six hundred subjects (264 men and 336 women) aged 65 to 102 years (mean, 79 ± 9 years) were randomly chosen from those admitted as inpatients to the Geriatric Division of the Estense Hospital of Modena (a city with a catchment area of about 300,000 inhabitants in Northern Italy) from January 1997 to January 2000.

In all patients, weight and height were assessed at the time of admission, and body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. On the morning of the following day, after a 12-hour overnight fast, venous blood was drawn to determine biological nutritional parameters such as plasma cholesterol,[20] triglycerides,[21] total proteins,[22] albumin,[23] and lymphocyte count; the patients were also assessed for plasma creatinine,[24] and homocysteine and serum vitamin B_6, vitamin B_{12} and folate.

Creatinine clearance was calculated using the Crockott-Gould formula.[25] All patients were also submitted to an oral methionine load

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(100 mg/kg), and after 4 hours a blood sample was drawn to measure homocysteine plasma levels.

Total plasma Hcy was assayed by high-performance liquid chromatography (HPLC) with fluorimetric detection, using cysteamine as internal standard for better quantitative determination.[26] Serum vitamin B_6 was assayed as pyridoxal-5-phosphate (PLP) by an HPLC commercial kit (Chromsystem Instruments and Chemicals GmbH, München, Germany); serum folate and vitamin B_{12} by enzyme-linked immunsorbent assay (ELISA) commercial kits (Abbott Lab, Abbott Park, IL).

All patients were also evaluated for body muscle mass by an indirect anthropometrical measurement, the mid-upper arm muscle area (MUAMA); it was calculated by the following equation: MUAMA = [mid-upper arm circumference (cm) – (triceps skinfold thickness (cm) $\times$ 3.14)/4π]; the result was corrected by -10 or - 6.5 if the subject was male or female, respectively.[27]

In addition, the presence of the following diseases (ICD-9-CM according to 1997 modification)[28] was recorded upon hospital admission and discharge: heart failure (ICD-9 428, n = 246), renal failure (ICD-9 584-586, n = 246), malignancies (ICD-9 140 to 239, n = 130), chronic obstructive lung disease (ICD-9 490-496, n = 252), dementia (ICD-9 290, n = 155), heart ischemic disease (ICD-9 410 to 414, n = 135), cerebrovascular disease (ICD-9 430 to 438, n = 157), peripheral vascular disease (ICD-9 440 to 448, n = 152), diabetes (ICD-9 250, n = 138), and inflammatory bowel disease (ICD 9 555 to 556, n = 23). Malnutrition (estimated energy intake $<$ 600 cal/d for $\geq$1 week before admission, n = 105) and chronic use of diuretics (n = 320), corticosteroids (n = 35), anticonvulsants (n = 78), or other drugs known to influence homocysteine metabolism (n = 15) over the 2 weeks prior to admission were also recorded.[29,30]

Hyperhomocysteinemia was diagnosed when fasting plasma tHcy concentration was at least 15 μmol/L (fasting hyperhomocysteinemia) and/or tHcy concentration 4 hours after a methionine load exceeded 35 μmol/L. We used the methionine load to increase the sensitivity of diagnosing homocysteine metabolism disturbance; according to Bostom et al, this test is able to identify a sizable percentage ($>$30%) of persons who may have clinically relevant hyperhomocysteinemia.[31]

The cut-off value for "methionine intolerance" was set at a 4-hour tHcy value 2 SD higher than the average for a control population of 300 healthy subjects, according to a criterion adopted by several authors.[7,32,33]

The data were analyzed using the following statistical tests: unpaired Student's *t* test, one-way analysis of variance (ANOVA), chi-square test, simple linear regression analysis, and stepwise multiple regression analysis; analysis of covariance performed by means of the GLM program (SPSS, Chicago, IL) to obtain and compare tHcy values corrected for vitamin status and/or creatinine clearance within groups. Statistical analysis were performed using SPSS statistical software (Chicago, IL). Data are presented as the mean $\pm$ SD; *P* values less than .05 were considered statistically significant.

RESULTS

Table 1 shows the main clinical and biochemical features of the subjects. The mean fasting tHcy level was 16.8 $\pm$ 12 μmol/L in the whole sample, 18.18 $\pm$ 13.25 μmol/L in men, and 15.86 $\pm$ 12.14 μmol/L in women (P = .005 men *v* women). Mean tHcy level 4 hours after methionine load was 37.95 $\pm$ 20.9 μmol/L in the whole sample. No significant differences were observed within sex (data not shown) with exception for BMI (23.5 $\pm$ 3.1 *v* 24.6 $\pm$ 4.2, $P < .05$ women *v* men) and creatinine clearance (47.2 $\pm$ 18.7 *v* 52.5 $\pm$ 19.6 mg/dL, women *v* men, $P < .05$). The frequency distribution for plasma fasting tHcy is reported in Fig 1. A few subjects (n = 7; 3 men and 4 women) with very low fasting homocysteine plasma levels ($<$6.0 μmol/L). All took very high daily supplements of folate and vitamins B_6 and B_{12}.

Table 1. Simple Correlation Coefficients for Plasma Homocysteine and the Indicated Variables in the Pooled Studied Population

Parameter	Mean Values (±SD)	Correlation Index With Fasting tHcy (*R*)	Correlation Index With 4-h tHcy (*R*)	Correlation Index With tHcy Change¶ (*R*)
Age (yr)	79 ± 9	.503*	.486*	.391*
Serum folate (ng/mL)	3.9 ± 3.6	−.485*	−.461*	−.221†
Vitamin B_6 (PLP) (ng/mL)	5.2 ± 4.1	−.134†	−.327*	−.325*
Vitamin B_{12} (pg/mL)	425 ± 221	−.274*	−.218*	−.185†
BMI (kg/m^2)	23.9 ± 3.8	−.089	−.100	−.125
MUAMA (cm^2)	37.5 ± 11.1	−.200†	−.201†	−.190†
Plasma albumin (g/dL)	3.6 ± 2.3	−.215†	−.219†	−.205†
Total plasma proteins (g/dL)	6.6 ± 0.6	−.171†	−.171†	−.166‡
Lymphocyte count (μL)	1,665 ± 701	−.148‡	−.140§	NS
Creatinine clearance (mL/min)	47.2 ± 18.2	−.286*	−.290*	−.250*
Total cholesterol (mg/dL)	190 ± 52	NS	NS	NS
Triglycerides (mg/dL)	125 ± 55	NS	NS	NS

*$P < .01$; † $P < .05$; ‡ $P = .058$; § $P = .061$; NS, not significant (vitamins normal range: folate 2 to 20 ng/mL; B_6 3.6 to 18 ng/mL; B_{12} 160 to 959 pg/mL).

¶Difference between tHcy 4 hours after methionine load and fasting tHcy.

HHcy was found in about 61% of subjects (365/600) (67% in men and 56% in women, $P < .05$). HHcy was rarely (8%) isolated; besides diabetes (20%), renal failure (48.2%), malnutrition (20.2%), heart failure (30%), malignancies (20.5%), and diuretics (56%) and anticonvulsant drug intake (13%), it was also associated with obesity (22%) and hyperlipemia (25%).

Plasma tHcy progressively increased across subjects from those with no diabetes, malnutrition, renal failure, obesity, inflammatory bowel disease, heart failure, etc, to those with 1, 2, or more concurrent diseases (Fig 2).

Table 2 shows tHcy plasma levels (mean $\pm$ SD) according to concurrent disease and drug use in the whole sample: tHcy was significantly higher in subjects with definite vascular atherosclerotic diseases, dementia, and malnutrition, even after correction for renal function (creatinine clearance) and vitamin status; and after correction for vitamin status in those with renal failure. After correction for vitamin status and renal function, the difference in tHcy levels in the presence or absence of heart failure, diabetes, and inflammatory bowel disease was lost, though remaining close to statistical significance. Also patients taking diuretics, anticonvulsants, and other drugs known to interfere with Hcy metabolism showed higher mean tHcy levels, which remained significantly higher after correction for vitamin status and creatinine clearance in those taking diuretics

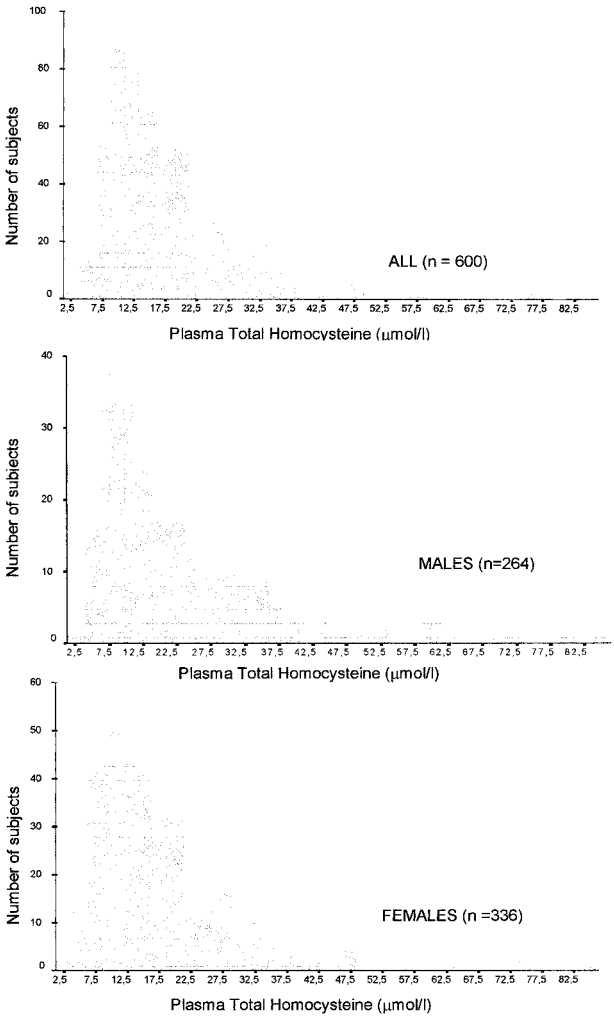

Fig 1. Frequency distribution for fasting plasma homocysteine levels in pooled subjects and in men and women separately.

or other tHcy-interfering drugs. Similar results were obtained after stratification for sex (data not shown).

HHcy was more prevalent in patients with definite vascular disease (n = 335) (69.5% *v* 21.7%, $P < .001$) and in those with cognitive impairment (diagnosis of probable dementia) (n =155) with respect to nondemented patients (63% *v* 32%, $P < .001$); moreover, patients with malnutrition (71.4% *v* 49.1%, $P < .01$), diabetes (51.3% *v* 41.2%, $P < .01$) and inflammatory bowel disease (66.4% *v* 53.2%, $P < .05$) had a significantly higher prevalence of HHcy; likewise patients taking anticonvulsants and diuretic drugs had a significantly higher prevalence ($P < .01$ each) of HHcy (Table 2). The prevalence of HHcy did not reach the statistical significance after correction for vitamin status and renal function between patients with or without heart failure ($P = .091$), whereas it remained close to statistical significance ($P = .052$ and $P = .056$, respectively) in case of presence or absence of diabetes and inflammatory bowel disease. After correction for vitamin status and creatinine clearance there was no modification of the significant higher prevalence (20% *v* 9.3%, $P < .01$) of HHcy in patients with hyperlipemia, whereas significance was lost between obese and non-obese patients (15% *v* 13%, $P = .081$).

The association between HHcy and vascular disease, expressed as the odds ratio, proved to be significant with a value of 2.5 (95% confidence interval [CI], 1.45 to 3.26). The association of HHcy with dementia was also significant, with an odds ratio of 5.02 (95% CI, 3.1 to 8.11).

Simple linear regression analysis (Table 1) showed a significant association of fasting (and 4 hours after methionine load) tHcy concentration with age, serum folate, vitamins B_6 and B_{12}, MUAMA, plasma albumin and total protein levels, and renal function (measured as creatinine clearance). These correlations remained significant both in pooled subjects and separately in women and men (data not shown).

Multiple stepwise regression (Table 3) indicated that 72% of plasma total fasting homocysteine variability was explained by 5 variables: age, serum folate, plasma albumin, creatinine clearance and use of diuretics. The other variables considered (MUAMA, PLP serum levels, presence of disease such as heart failure or malignancies), although significant independent predictors of plasma fasting homocysteine, only marginally explained its variability.

When tHcy level 4 hours after methionine oral loading was taken as the independent variable, 76% of its variability is explained by 6 variables (5 are the same observed for fasting homocysteine variability, the fifth being B_6 serum levels) (data not shown).

DISCUSSION

In this study, we found that the mean plasma tHcy levels in a large sample of elderly hospitalised subjects was 16.8 ± 12 μmol/L, about 30% to 40% higher than that reported in other epidemiological studies.[4,15-18,34] It is likely that this difference is strongly influenced by the higher co-morbidity and older age of our case series. Probably for the same reason, the prevalence of fasting HHcy (48% in our pooled sample) was higher than that reported by others.[4,16,17,34] The prevalence of overall disturbance of homocysteine metabolism (hyperhomocysteinemia

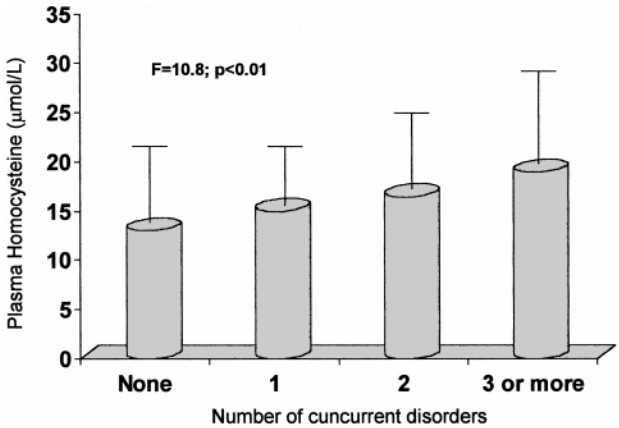

Fig 2. Fasting plasma homocysteine concentration (mean ± SD) in relation to associated concurrent diseases (ie, obesity, malnutrition, diabetes mellitus, hyperlipaemia, renal failure, inflammatory bowel disease, malignancies) in 600 elderly hospitalized patients.

Table 2. Fasting Homocysteine Concentration and Prevalence of HHcy According to Concurrent Disease and Drug Use in the Pooled Sample

Disease/Drug	Present	Absent	P‡
Disease			
Heart failure (n = 246)	17.8 ± 5.6	16.3 ± 7.5	<.05
	(45%)	(42%)	**.061**[a]
Atherosclerotic disease*	18.7 ± 7.4	14.4 ± 6.2	<.01
(n = 335)	(69.5%)	(21.7%)	**<.05**[a]
Renal failure (n = 246)	19.4 ± 8.2	14.9 ± 7.2	<.01
	(71.7%)	(55.1%)	**<.05**[b]
Diabetes (n = 138)	17.1 ± 8.4	16.71 ± 9.2	.055
	(51.3%)	(41.2%)	**.058**[a]
Malignancies (n = 130)	17.2 ± 4.1	16.6 ± 6.8	<.05
	(57.2%)	(50.2%)	**<.05**[a]
Malnutrition (n = 105)	19.3 ± 7.3	16.3 ± 6.5	<.01
	(71.4%)	(49.1%)	**<.05**[a]
Dementia (all types) (n = 155)	18.9 ± 8.2	16.1 ± 7.3	<.01
	(63%)	(32%)	**<.05**[a]
Inflammatory bowel disease	17.3 ± 5.8	16.8 ± 8.9	<.05
(n = 23)	(66.4%)	(53.2%)	**.058**[a]
Drug			
Diuretics (n = 320)	17 ± 10.2	16.5 ± 7.8	<.05
	(65.3%)	(52.3%)	**<.05**[a]
Corticosteroids (n = 35)	16.4 ± 15.2	16.8 ± 11.4	NS
	(62.3%)	(55.5%)	**NS**
Anticonvulsants (n = 78)	17.5 ± 8.7	16.7 ± 8.1	<.05
	(61.7%)	(52.8%)	**.054**[a]
Others† (n = 15)	17.6 ± 10.5	16.8 ± 11.3	<.05
	(60.3%)	(48.3%)	**<.05**[a]

* Comprising patients with 1 or more documented atherosclerotic diseases (cardiac, cerebrovascular, or peripheral arterial disease).

† Comprising drugs such as methotrexate (n = 1), isoniazide (n = 3), sulfamides (n = 3), penicillamine (n = 3), and drugs interfering with bile acids absorption (n = 5).

‡ Bold *P* values are results after correction (analysis of covariance) for [a] creatinine clearance and vitamin status (serum folate, vitamins B_{12} and B_6 levels) and [b] vitamin status only.

either fasting or after methionine loading) in our population was 61%, 13% higher than that detectable when only referring to fasting values; this partially confirms data on the importance of methionine loading for identifying persons with disorders of homocysteine metabolism.[7,31,32]

Higher comorbidity and older age may also be important for explaining data on homocysteine-related vitamin status of our population: even if within the normal range, serum vitamin levels showed in fact a relatively high variability (Table 1).[35]

Consistent with other reports, our population presented a significant difference in mean plasma Hcy concentration within sexes.[17] Besides sex differences in vitamin status (a difference we did not observe in our study),[16,36] it may depend on different Hcy production from methionine demethylation and creatine-creatinine synthesis.[16-17,36] On the other hand, a possible influence of sex hormones has been suggested,[16,37-39] and, although most of women examined in the present study had been postmenopausal state for a long time, their blood testosterone to oestradiol ratio may be thought to be lower than that of men.[40]

In many individuals with HHcy suffering from diseases or conditions known to influence homocysteine metabolism (eg, renal failure or malnutrition), it is hard to exclude the concomitant presence of primary abnormalities of homocysteine metabolism. However, in our study the overall prevalence of homocysteine metabolism disturbance (ie, HHcy at fasting or after methionine load) was 61%, by far higher than the prevalence of the main genetic defects known to induce mild HHcy (ie, 1% to 2% heterozigotes for cystathionine-β-synthase deficit, and 12% to 15% for methylenetetrahydrofolate reductase mutation).[41-43]

In our study, HHcy was frequently (Table 2) associated with other concurrent diseases such as diabetes, malnutrition, renal failure, inflammatory bowel disease, and hyperlipidemia. In particular, the higher prevalence (approaching statistical significance even after correction for creatinine clearance) of HHcy in patients with diabetes is consistent with the results obtained by other investigators and suggests a possible link between insulin metabolism disturbance and homocysteine metabolism.[44,45] The association with inflammatory bowel disease is interesting as well: our data suggest that it depends mostly on vitamin status (absorption impairment); it may be relevant in explaining the higher prevalence of thromboembolism in patients affected by inflammatory bowel disease.[46] On the other hand HHcy was also more frequent in patients with hyperlipidemia, an important risk factor for vascular disease. The correlation between homocysteine and lipid metabolism is still unclear, although some investigators have reported a reduction in plasma homocysteine levels in patients taking lipid-lowering drugs[47] and a possible role of lipopoproteins in plasma Hcy binding.[48]

Moreover, the relationship between diuretics and homocysteine is consistent with other reports.[29] It is known that these drugs can influence tubular secretion and/or increase tubular absorption of homocysteine, thus reducing its renal excretion. On the other hand, diuretics are commonly used by patients affected by clinical syndromes such as chronic renal failure or heart failure, which in our populations (Table 2) were associated with higher tHcy. Similarly anticonvulsants may influence

Table 3. Multiple Stepwise Regression Analysis: Standardized Regression Coefficients for Fasting Homocysteine Concentration (as dependent variable) on Indicated Covariates (N = 600)

Covariate	Standardized Regression Coefficient	F	P
Age	.45	78.9	.000
Serum folate	−.35	71.4	.001
Creatinine clearance	−.31	63.1	.001
Diuretics*	−.21	48.9	.019
Albumin	−.16	17.5	.032
	R^2 = .718		

NOTE. The model also included the following variables: serum vitamins B_{12} and B_6, BMI, MUAMA, lymphocyte count, heart failure, malignancies, inflammatory bowel disease, and anticonvulsants. Instead of malnutrition, we used anthropometric and plasma nutritional markers, and instead of renal failure, we used creatinine clearance (continuous values).

* Parametric value.

homocysteine metabolism via their effect on folate metabolism[30]: this agrees with the loss of significance after controlling for vitamin status (Table 2).

The significant prevalence of HHcy in patients with documented atherosclerotic disease confirms the different prospective studies reporting a higher relative risk of vascular disease in patients with higher than normal plasma homocysteine concentrations,[5-7,49] suggesting a role for homocysteine in triggering the development of thrombosis and atherosclerosis. On the other hand, Dudman recently suggested a positive role for homocysteine in vascular disease: its increase might be a consequence of damage, and its release from damaged vascular tissues may provide some kind of benefit by promoting tissue repair.[50]

Correlation statistics (both simple linear regression and multiple regression analysis) underline the importance of nutritional and particularly of vitamin (folate, vitamins B_6 and B_{12}) status in inducing HHcy. It is well known that the elderly population is at high risk of malnutrition, and especially of vitamin deficiency.[1-4,14-18] In our population mean levels of serum vitamins fall within the "normal" range (Table 1); nevertheless we noticed a relatively high prevalence of subjects with deficit (serum levels lower than normal) of 1 (31%), 2 (21%), or 3 (18%) homocysteine-related vitamins. Moreover, a great portion (67%) of our population had one or more vitamin levels near (within 15% higher) the lower recommended range limit, suggesting the need for a review for reference values in elderly people.[17,51]

Interestingly, our data confirm the association between HHcy and cognitive impairment (dementia), as already postulated by others. This may depend both on a vascular effect and a direct toxic neuronal effect by homocysteine or some metabolites known to be toxic for neurons in vitro.[8-13] Nevertheless it should be kept in mind that vitamin B_{12}, vitamin B_6, and folate deficits (of which mild hyperhomocysteinemia may be an early marker) are often associated with neurological disturbances and poor performance on neuropsychological tests, especially in the elderly.[8,10-12] The role of HHcy per se or as an expression of vitamin deficit in inducing neurological disturbances is still undefined and merits further investigations; even if it is not the aim of our work, it is important to note that in our population (Table 2) the difference of mean tHcy levels and the prevalence of HHcy remained significantly higher in patients with dementia, even after correction for vitamin status and creatinine clearance (Table 2).

Multiple stepwise regression analysis documented that beyond age, creatinine clearance and nutritional status (and particularly folate vitamin status) are strong predictors of increased plasma homocysteine levels. This finding confirms the close relationship between vitamin intake and plasma homocysteine, especially in the elderly,[2,3] and may indicate a possible target for therapeutic intervention.

In conclusion, the present study documents that in elderly hospitalized subjects, HHcy is (1) a common finding, (2) frequently associated with vascular and cognitive disorders, and (3) probably a secondary phenomenon in most cases. The major predictors of homocysteine metabolism disturbances (high homocysteine plasma levels fasting or after methionine load) are age, renal function, nutritional status (serum folate and plasma albumin), and the use of some drugs such as diuretics. Overall, these factors explain more than 70% of fasting homocysteine variability.

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