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Plasma homocysteine concentration in children with chronic renal failure

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Abstract Hyperhomocysteinemia, a risk factor for vascular disease, is commonly found in adult patients with end-stage renal disease. Major determinants of elevated plasma homocysteine levels in these patients include deficiencies in folate and vitamin B_{12}, methylenetetrahydrofolate reductase (MTHFR) genotype and renal function. Little information is available for children with chronic renal failure (CRF). The prevalence and the factors that affect plasma homocysteine concentration were determined in children. Twenty-nine children with various degrees of CRF (15 were dialyzed, 14 were not dialyzed) were compared with 57 age- and sex-matched healthy children. Homocysteine concentrations were higher in patients than controls (17.3 µmol/l vs 6.8 µmol/l, $P<0.0001$) and hyperhomocysteinemia (>95th percentile for controls: 14.0 µmol/l) was seen in 62.0% of patients and 5.2% of controls. Folate concentrations were lower in patients (9.9 nmol/l) than controls (13.5 nmol/l), $P<0.01$. Vitamin B_{12} was similar in patients (322 pmol/l) and controls (284 pmol/l). Dialyzed patients have a higher prevalence of hyperhomocysteinemia than nondialyzed patients (87% vs 35%). Dialyzed patients with MTHFR mutation have higher plasma homocysteine (28.5 µmol/l) than nondialyzed patients with the mutation (10.7 µmol/l), $P<0.002$. In our study, differences between controls and patients in plasma homocysteine concentrations are observed when age is greater then 92 months, folate less than 21.6 nmol/l and vitamin B_{12} less than 522 pmol/l. Our study shows that hyperhomocysteinemia is common in children with CRF and is associated with low folate and normal vitamin B_{12} status, compared to normal children. Among the patients, the dialyzed patients with the MTHFR mutation are particularly at risk for hyperhomocysteinemia. Further studies are needed to investigate therapeutic interventions and the potential link with vascular complications in these patients.

Keywords Plasma homocysteine concentration · Vitamin B_{12} · Folic acid · MTHFR genotype · Chronic renal failure

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Introduction

Homocysteine is a thiol-containing amino acid derived from the metabolism of methionine. It is metabolized through the transsulfuration pathway by cystathionine-β-synthase (CBS) to form cysteine or remethylated to form methionine by methionine synthase (MS), a reaction that is dependent on the cofactor vitamin B_{12}. Methylenetetrahydrofolate reductase (MTHFR) synthesizes the folate derivative that provides the methyl group for the methionine synthase reaction.

Disorders of homocysteine metabolism resulting in hyperhomocysteinemia can be caused by genetic and nongenetic factors. Severe genetic deficiencies of CBS and MTHFR activity (homocystinuria) are rare. The most common form of genetic hyperhomocysteinemia results from production of a thermolabile variant of MTHFR with moderately reduced enzyme activity [1]. Homozygosity for this polymorphism occurs in 10%–12% of the general North American population and is associated with increased plasma homocysteine concentration in subjects with low folate [2]. Nongenetic causes of hyperhomocysteinemia include dietary deficiencies of folate and vitamin B_{12} [3] and chronic renal failure [4, 5].

Several studies have shown that homocysteine is an independent risk factor for vascular disease [6] in the general population. Elevated homocysteine levels have also been reported in adult patients with chronic renal failure undergoing chronic dialysis and in transplant patients [4, 5, 7, 8].

To date, pediatric data on children with CRF are limited [9]. We therefore studied the interactions between MTHFR genotype, folate, vitamin B_{12} status and uremia on the plasma homocysteine concentration in 29 pediatric patients with chronic renal failure and compared them with an age- and sex-matched population of healthy children.

Materials and methods

Patients and controls

Study population

The study population consisted of 29 patients with chronic renal failure (17 girls, 12 boys). Primary renal disease was urologic malformations in eight patients, cystinosis in seven patients, hereditary nephritis in three patients, dysplastic kidneys in two patients, hemolytic uremic syndrome in two patients, focal segmental glomerulosclerosis in two patients, IgA nephropathy in two patients, cortical necrosis in one patient, and unknown etiology in two patients. The duration of CRF for patients ranged from 6 months to 11 years (mean 4.61±3.61 years). Patients had various levels of chronic renal dysfunction. Fifteen patients were dialyzed (nine on peritoneal and six on hemodialysis); the median duration of dialysis treatment prior to the study was 20 months (range: 1 month to 125 months). Fourteen patients were not dialyzed.

Ethnic origin was Caucasian (27 patients), Asian (1 patient) and black (1 patient).

Patients remained on their normal diets. None of them received routine supplementation with folate and/or vitamin B_{12}. Nondialyzed patients were on moderate protein restriction.

Those with ESRD received high caloric, low protein, and low phosphate diets with sodium bicarbonate to correct metabolic acidosis, calcium carbonate as a phosphate binder to maintain the plasma phosphate concentration in the normal range and vitamin D supplements adjusted to control phosphate and calcium concentration in the normal range. Antihypertensive treatment was administered if necessary. No one was receiving prednisone or cyclosporine treatment. Cycled peritoneal dialysis was provided by an automated cycling machine (Fresenius) through a peritoneal Tenckhoff catheter; the dwelling volume was 40 ml/kg with seven to ten exchanges every night. Hemodialysis was performed 2–3 times a week using Cobe machines with bicarbonate solutions, 3–4 h/session. The patients were hemodialyzed through permanent vascular access (three had arteriovenous fistula, three had a permanent central catheter) using hollow fiber dialysers (range of the surface 0.6–1.7 m^2). Blood flow was 50–200 ml/min; dialysate flow was 500 ml/min. All patients were in stable condition without concurrent illness during the study.

Laboratory measurements

After the study was approved by the Ethics Committee of Saint-Justine Hospital and informed consent obtained from the parents of patients, the patients were asked to collect a 24-h urine and blood samples were drawn in the morning after an overnight fast. Patients on cycling peritoneal dialysis were asked to discontinue dialysis 12 h before sampling. A single blood sample was obtained from each patient for the determination of plasma total homocysteine, folic acid and vitamin B_{12}, red blood cell (RBC) folate, routine biochemistry analysis and MTHFR genotyping.

Total plasma homocysteine

Total plasma homocysteine was determined using high-performance liquid chromatographic and electrochemical detection [10]. After blood collection in EDTA-containing tubes, samples were centrifuged, and plasma was separated without delay and stored at –20°C until analysis. All forms of plasma homocysteine were determined including reduced and oxidized forms, all collectively referred to as total homocysteine. Hyperhomocysteinemia was defined as values above the 95th percentile for our control population (14.0 µmol/l).

Folic acid and vitamin B_{12} concentrations

Plasma vitamin levels were determined using blood samples drawn at the time of measurement of homocysteine. Plasma folate and vitamin B_{12} were measured by a double-labeled radioimmunoassay (Ciba-Corning). Deficiencies in plasma folate and vitamin B_{12} concentration were defined as values less than the 5th percentile for our control population (10.1 nmol/l and 160.2 pmol/l, respectively).

MTHFR genotype

Genotyping was performed by PCR amplification of genomic DNA extracted from blood leukocytes in all patients. The method has been described elsewhere [1]. We used TT, CT, and CC to refer to subjects who were homozygous for the mutation, heterozygous for the mutation and homozygous for the wild type, respectively.

Renal function determination

The serum creatinine concentration and the determination of glomerular filtration rate were used to assess renal function. Chronic renal failure was defined as persistently elevated serum creatinine for age and a fall in GFR of less than 80 ml/min/1.73 m^2. GFR was estimated by the calculated creatinine clearance using the age specific k values of Shwartz et al. [11]. In 24 patients, there was a highly significant positive correlation between the calculated creatinine clearances and those measured by 24-h urine collections (r=0.85, P<0.0001) (data not shown). We then used calculated creatinine clearance for nonanuric patients.

Creatinine, glucose, and albumin were measured using a standard laboratory technique on blood samples obtained at the same time as those for assay of homocysteine.

Controls

Fifty-seven healthy children (35 girls, 22 boys, age range: 30–217 months) served as age- and sex-matched controls. Blood samples were collected for the determination of plasma levels of total homocysteine, folic acid and vitamin B_{12} and for assessment of MTHFR genotype. The 57 healthy children were selected to match the study population from a previous study of control children [12].

Statistical analysis

Comparison of proportions was performed with Pearson's chi-square or Fisher's exact test, and the nonparametric comparison of groups was performed with the Kruskal-Wallis test for the determination of continuous variables. Comparison of means was performed with one-way ANOVA and adjusted for inequality of variances when appropriate. Linear regression analysis was calculated by the method of least squares when necessary. All tests were performed using SAS software.

Table 1 Characteristics of healthy controls, subjects and patients with CRF. Results are expressed as means ± SD (*tHcy* total plasma homocysteine)

	Patients (*n*=29)	Controls (*n*=57)	*P* value
Age (years)	12.4±4.5	11.5±4.8	NS
Sex (F/M), *n* (%)	17 (58.6%)/12 (41.4%)	35 (61.4%)/22 (38.6%)	NS
Weight (kg)	37.79±20.71	43.29±20.86	NS
Vitamin B_{12} (pmol/l)[a]	322 (75–1020)	284 (136–570)	NS
Folic acid (nmol/l)[a]	9.9 (4.4–49.1)	13.5 (9.4–26.5)	<0.01
tHcy (µmol/l)[a]	17.3 (5.89–137.8)	6.8 (3.07–24.34)	<0.0001
MTHFR genotype, *n* (%) (CC/CT/TT)	12 (41.4%)/13 (44.8%)/4 (13.8%)	26 (45.6%)/23 (40.4%)/8 (14.%)	NS

[a] Median (range)

Results

Patients and controls were not significantly different for age, gender and body weight. Table 1 shows plasma homocysteine, vitamin B_{12} and folate concentrations and MTHFR genotype frequencies in patients and controls.

Plasma homocysteine

Median plasma homocysteine concentration was significantly higher in patients than controls (17.3 µmol/l vs 6.8 µmol/l, $P<0.0001$). Hyperhomocysteinemia was found in 62.0% of children with CRF compared to 5.2% of controls ($P<0.001$).

Vitamin B_{12}

Median vitamin B_{12} concentrations were not significantly different between patients and controls (322 pmol/l vs 284 pmol/l). Deficiency was seen in four patients (13.8%) and three controls (5.3%) ($P=0.39$).

Folate status

Median plasma concentration of folate was significantly lower in patients (9.9 nmol/l) than in controls (13.5 nmol/l) ($P<0.01$) and folate deficiency was seen in 17 patients (58.6%) compared with 4 controls (7.0%) ($P<0.001$).

MTHFR genotype

There was no significant difference in the frequency of MTHFR genotypes between CRF patients and controls (Table 1).The mutation was not present (CC) in 12 patients (41.4%) and in 26 controls (45.6%). Heterozygosity for the mutation was found in 13 patients (44.8%) and 23 controls (40.4%). Homozygosity for the mutation (TT) was found in four patients (13.8%) and eight controls (14.%).

Table 2 Plasma homocysteine concentration (tHcy) in patients and controls with CC, CT and TT MTHFR genotypes. Results are expressed as median and range

MTHFR genotypes	tHcy (controls) (µmol/l)	tHcy (patients) (µmol/l)	*P* value
CC	7.7 (3.07–14.86)	18.9 (7.44–92.41)	<0.001
CT	6.5 (3.44–13.67)	15.6 (5.89–50.12)	<0.001
TT	6.5 (3.60–24.30)	60.7 (12.6–137.8)	<0.01

Table 3 Relationship between homocysteine and age, plasma folate and vitamin B_{12} in patients and controls. Statistical analysis consisted of analysis of variance using the least mean squares method. Results for tHcy are expressed as means ± SEM

	tHcy (µmol/l)	*P* value
Folate (nmol/l)		
11.0	23.4±4.5	0.0001
13.4	21.1±3.9	0.0001
16.2	18.4±6.5	0.0001
21.6	13.0±6.5	0.05
Vitamin B_{12} (pmol/l)		
245	28.0±4.5	0.0001
295	25.6±4.0	0.0001
391	21.4±4.5	0.0001
522	14.5±7.2	0.05
Age (months)		
87	9.8±5.6	0.08
92	10.7±5.4	0.05
161	23.0±4.0	0.0001
191	28.3±5.0	0.0001

Table 2 shows a significantly higher plasma homocysteine concentration in CC, CT and TT patients than in CC, CT and TT in controls ($P<0.001$, $P<0.01$).

Linear regression analysis

As shown in Fig. 1, plasma homocysteine concentration increases with age in patients and controls. However, the difference becomes statistically significant in ages higher than 92 months (Table 3). Figure 2 shows that plasma homocysteine concentration decreases with increasing vitamin B_{12}; the increase in vitamin B_{12} up to

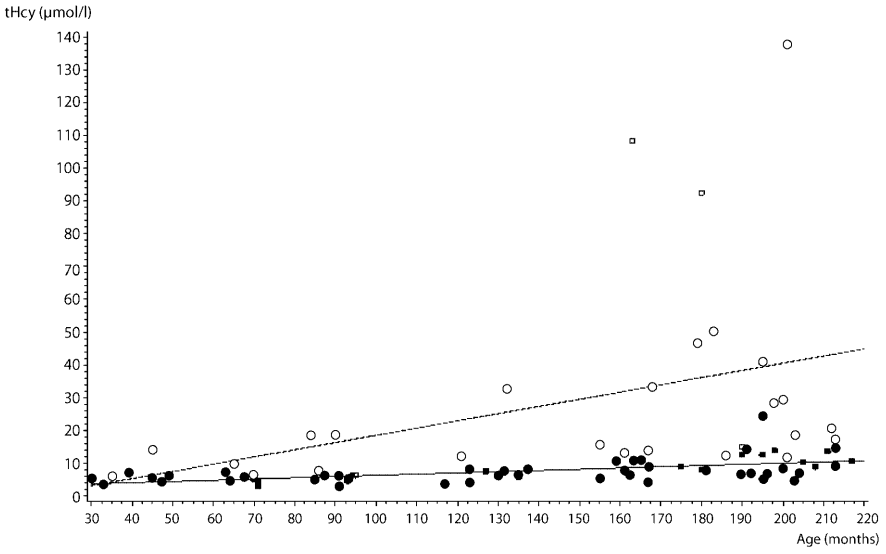

Fig. 1 Regression analysis between plasma homocysteine concentration and age in patients (□) and controls (●). A positive correlation was found in controls (r=0.58, P=0.0001) and patients (r=0.37, P=0.04). When age is divided into quartiles in patients and controls, statistically significant differences in plasma homocysteine concentration are observed at ages greater than 92 months

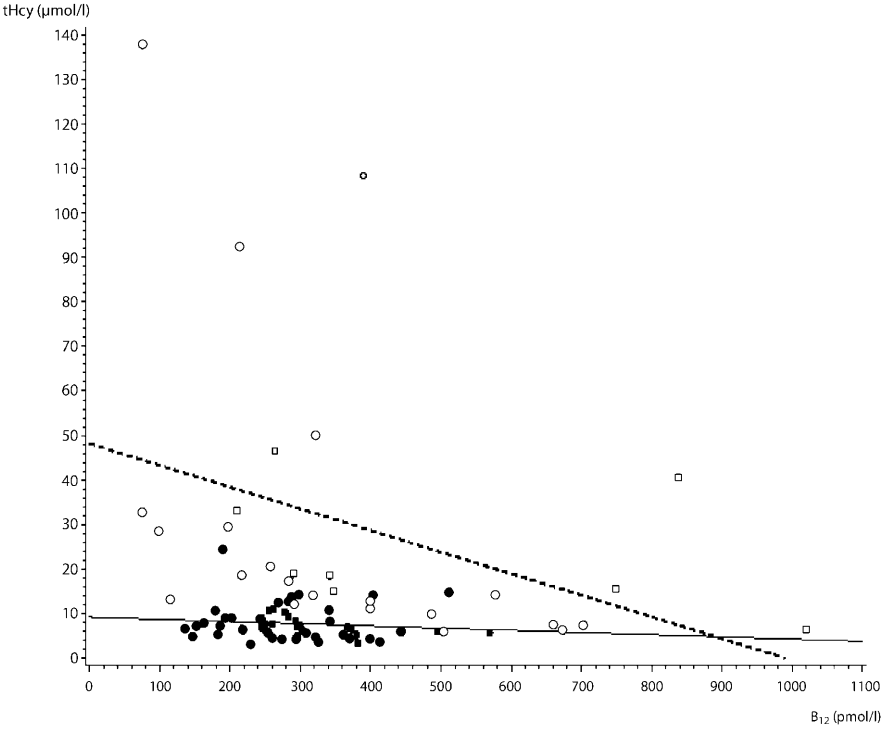

Fig. 2 Regression analysis between plasma homocysteine concentration and vitamin B_{12} in patients (□) and controls (●). A negative correlation was found in controls (r=–0.12, P=0.35) and patients (r=–0.36, P=0.05). When vitamin B_{12} is divided into quartiles in patients and controls, statistically significant differences in plasma homocysteine concentration are observed when vitamin B_{12} is lower than 522 pmol/l

522 pmol/l is statistically significant (Table 3). Figure 3 shows that plasma homocysteine concentration decreases significantly with folate up to levels of 21.6 nmol/l (Table 3).

Dialyzed and nondialyzed patients

Table 4 shows comparisons between dialyzed and nondialyzed patients. Patients were not significantly different for age, folate and vitamin B_{12} status, serum albumin and MTHFR genotype frequencies. A significantly higher median plasma homocysteine concentration was seen in dialyzed patients than in nondialyzed patients (28.5 µmol/l vs 12.3 µmol/l, P<0.004). Figure 4 shows the prevalence of hyperhomocysteinemia in dialyzed patients, nondialyzed patients and controls.

Hyperhomocysteinemia was seen in 86.6% of patients undergoing dialysis (median GFR: 5.3 ml/min/1.73 m^2) and in 35.7% of patients with mild to moderate CRF (median GFR: 27.0 ml/min/1.73 m^2) (P<0.001). Pooled dialysis patients with the CT+TT MTHFR genotype had a significantly higher plasma homocysteine concentration than nondialyzed patients with the CT+TT genotypes (28.5 µmol/l vs 10.6 µmol/l, P<0.002) (Table 4).

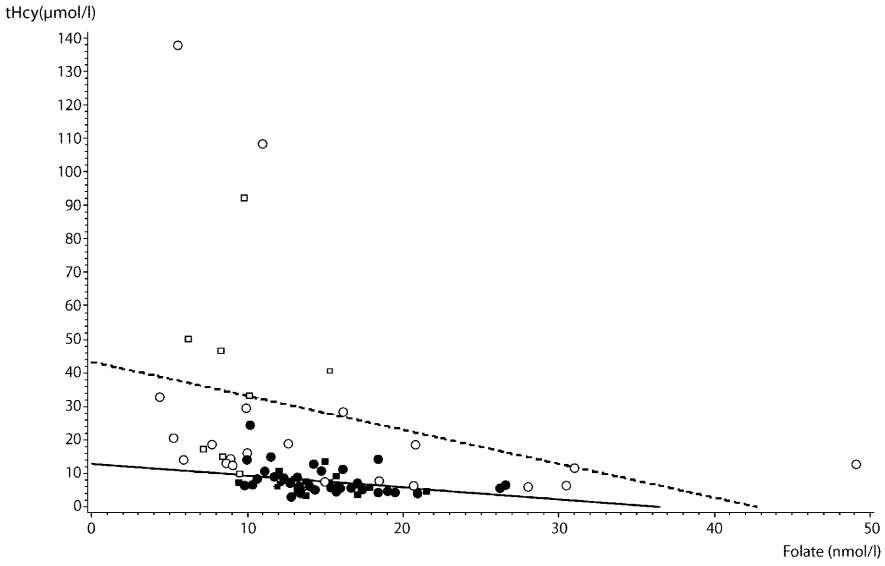

Fig. 3 Regression analysis between plasma homocysteine concentration and folate in patients (□) and controls (●). A negative correlation was found in controls (r=–0.34, P=0.009) and patients (r=–0.31, P=0.09). When folate is divided into quartiles in patients and controls, statistically significant differences are observed when folate is lower than 21.6 nmol/l

Table 4 Comparison between dialyzed and nondialyzed patients. Results are expressed as means ± SD

	Dialyzed patients (n=15)	Nondialyzed patients (n=14)	P value
Age (years)[a]	15 (7–17)	11 (3–18)	NS
tHcy (µmol/l)[a]	28.5 (12.1–137.8)	12.3 (5.9–92.4)	<0.004
Vitamin B_{12} (pmol/l)[a]	291 (75–938)	443 (75–1020)	NS
Plasma folate (nmol/l)[a]	9.9 (5.5–49.1)	12.4 (4.4–31)	NS
Albumin (g/l)	35.8±4.3	38.5±8.52	NS
RBC folate (nmol/l)[a]	747 (198–9520)	644 (244–1845)	NS
Creatinine (µmol/l)	964 (287–1339)	175 (71–555)	<0.0001
GFR (ml/min/1.73 m^2)[a]	5.28 (0–22)	26.56 (11–78)	<0.0001
tHcy (µmol/l)[a]/MTHFR			
CT+TT	28.5 (12.6–137.8)	10.6 (5.9–18.5)	<0.002
CC	17.31 (12.1–40.5)	17.2 (7.4–92.4)	NS
MTHFR genotype, n (%) (CC/CT/TT)	6 (40)/6 (40)/3 (20)	6 (42.9)/7 (50)/1 (7.1)	NS

[a] Median (range)

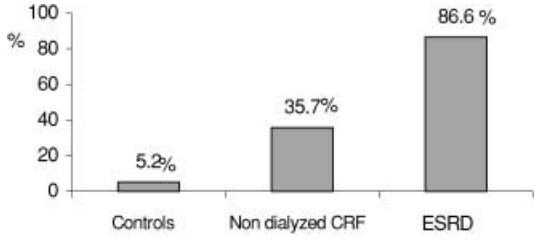

Fig. 4 Hyperhomocysteinemia was found in 5.2% of controls, 35.7% of nondialyzed CRF patients and 86.6% of dialyzed CRF patients (ESRD) (P<0.001)

Discussion

In the present study, we analyzed the prevalence and determinants of elevated plasma homocysteine levels in pediatric patients with CRF. Elevated plasma homocysteine has been identified as a significant risk factor for various forms of vascular disease in the general population [6] and in patients with ESRD [4, 13, 14, 16]. Hyperhomocysteinemia is usually found in nearly 60–90% of adult patients with ESRD [4, 5, 15]. The present study shows that children also have a higher prevalence of hyperhomocysteinemia (65.5%) than the normal pediatric population (5.2%).

Although the exact cause of hyperhomocysteinemia in CRF is unknown, numerous studies performed in adult patients with ESRD identified folate, vitamin B_{12}, MTHFR genotype and renal function as major determinants of plasma homocysteine concentration [2, 17, 18] in that setting. We therefore analyzed these factors in children with CRF and compared them with a normal pediatric population.

The kidney has an important role in the metabolism of homocysteine metabolism and a markedly reduced clearance is associated with a rise in plasma homocysteine concentration [19–21]. Despite the limitation of a relatively small number of patients, median plasma homocysteine concentration was higher in patients with

CRF compared to controls. These findings are compatible with previous studies of CRF patients [4, 22]. The present study demonstrates that plasma homocysteine is already significantly elevated in nondialyzed CRF patients (12.3 µmol/l) as compared to controls (6.8 µmol/l), and increases gradually with the deterioration of renal function and the need for dialysis to a fourfold increase (28.5 µmol/l). In agreement with other studies [17], our dialyzed patients had a higher plasma homocysteine concentration and a higher prevalence of hyperhomocysteinemia (86.6%) than nondialyzed patients (35.7%).

Folate status is a major determinant of plasma homocysteine in patients with ESRD. There is a strong inverse relationship between the plasma homocysteine concentration in normal individuals and patients with ESRD [23]. The plasma folate concentration is usually increased in patients because of routine supplementation with folate. Supplementation is required since other studies have shown decreased serum folic acid concentration in chronic dialysis patients. Patients with CRF are at risk of vitamin deficiencies, due to poor dietary intake because of dietary restriction and to the loss of folic acid during dialysis [24]. Our patients were not routinely supplemented and had a lower plasma folic acid concentration than normal children. Folate deficiency was found in over 58.6% of patients compared to 7.0% of controls. Folic acid is the most powerful plasma homocysteine lowering agent. Plasma homocysteine can also be modulated in CRF by plasma vitamin B_{12} concentration, although hyperhomocysteinemia persists in patients despite normal to supranormal levels of vitamin B_{12} [25]. In our study, the patients had normal plasma vitamin B_{12} concentrations compared to controls; deficiency in vitamin B_{12} was less common than folate deficiency and was seen in 13.8% of patients versus 5.3% in controls.

Homozygosity for the MTHFR mutation is also an important determinant of plasma homocysteine concentration in ESRD patients [26]. As homozygosity for the mutant MTHFR genotype was found in only a small number of patients, the study of an interaction between plasma homocysteine and homozygosity for the mutant MTHFR genotype was limited. We therefore pooled CT+TT patients and compared them with CC patients and found that plasma homocysteine was higher in dialyzed patients with the mutation.

In accordance with many studies, dialyzed patients on ESRD have a higher incidence of hyperhomocysteinemia than patients with mild to moderate CRF [4]. In contrast to studies on adult dialyzed patients where high folate concentrations were found due to supplementation [27, 28], in this study supplementation was not routine and we found no difference in folate status and vitamin B_{12} concentration in dialyzed and nondialyzed patients.

Age is a strong determinant of plasma homocysteine concentration as previously reported in normal children [12, 29]. In our previous study, we found a pronounced age dependence of plasma homocysteine in controls [12].

In this study, linear regression analysis showed that homocysteine differences between controls and patients are observed when age is greater than 92 months, folate less than 21.6 nmol/l and vitamin B_{12} less than 522 pmol/l.

Conclusions

We conclude that hyperhomocysteinemia is a common finding in children with CRF. Homocysteine lowering treatment should be considered in children older than 92 months, with low folate and vitamin B_{12} status, who are more likely to have hyperhomocysteinemia than controls. In patients, dialyzed patients with the mutant MTHFR genotype have higher homocysteine concentrations. Further studies are required to investigate therapeutic interventions and the potential link with vascular complications in patients with CRF.

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LITERATURE ABSTRACT

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Arginine, citrulline, and nitric oxide metabolism in end-stage renal disease patients

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The kidneys are thought to be a major site of net de novo arginine synthesis, but the quantitative status of arginine metabolism and its substrate precursor relationship to nitric oxide (NO) synthesis in end stage renal disease (ESRD) patients have not been characterized. We have investigated kinetic aspects of whole body arginine metabolism in six patients with ESRD. They received two pre- and two post-hemodialysis intravenous tracer infusion studies with L-[guanidino-(15)N(2)]arginine and L-[(13)C]leucine during the first study, and L-[5-(13)C]arginine and L-[5-(13)C-ureido,5,5,(2)H(2)]citrulline during the second study. Arginine homeostasis in ESRD patients was found to be associated with a lower rate of arginine oxidation, and despite the decrease in renal function, the rate of de novo arginine synthesis appeared to be preserved. Plasma citrulline concentrations and flux were also elevated in these subjects compared with healthy adults. The rate of whole body NO synthesis was increased in the ESRD patients, but apparently not different pre- and post-hemodialysis therapy. The anatomic site(s) responsible for the maintenance of net de novo arginine synthesis and for the elevated NO synthesis and its pathophysiological importance in ESRD remain to be established.