Aicha Merouani · Marie Lambert · Edgar E. Delvin
Jacques Genest, Jr. · Pierre Robitaille · Rima Rozen

Plasma homocysteine concentration in children with chronic renal failure

Received: 26 January 2001 / Revised: 16 April 2001 / Accepted: 17 April 2001

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\textsubscript{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\textsubscript{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 than 92 months, folate less than 21.6 nmol/l and vitamin B\textsubscript{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\textsubscript{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\textsubscript{12} · Folic acid · MTHFR genotype · Chronic renal failure

Introduction

Homocysteine is a thiol-containing amino acid derived from the metabolism of methionine. It is metabolized through the transsulfuration pathway by cystathionine-\(\beta\)-synthase (CBS) to form cysteine or remethylated to form methionine by methionine synthase (MS), a reaction that is dependent on the cofactor vitamin B\textsubscript{12}. Methylentetrahydrofolate 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\textsubscript{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. No one was receiving prednisone or cyclosporine treatment. No one was receiving prednisone or cyclosporine treatment.

**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 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 (14.0µmol/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 homoyzoygous 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 m². GFR was estimated by the calculated creatinine clearance using the age specific k values of Schwartz 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.