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Homocysteine Clearance in Hemodialysis

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Abstract

Background and Aims: Homocysteine is a sulphur amino acid derived from methionine. Epidemiological studies show an association between hyperhomocysteinemia and an increased cardiovascular risk, a fact that has also been confirmed in patients with chronic renal failure. This study, conducted in stage 5 chronic kidney disease patients, seeks to define the prevalence of hyperhomocysteinemia, evaluate the clearance of homocysteine with dialysis, and describe the frequency of the mutation in the mutilen-tetrahydrofolate reductase enzyme and its relationship with plasma levels of homocysteine.

Methods: The reduced percentage of homocysteine and clearance of urea were analysed every six months for seven years in patients on dialysis. Urea and total homocysteine in plasma were measured in each of these studies and the type of dialyser - low or high permeability - used and the dialysis duration was determined. A molecular study of the gene coding for mutilen-tetrahydrofolate reductase enzyme was carried out in a group of patients and any C-T point mutation at position 677 of this gene was investigated. Mutation was described as not present, heterozygous for this mutation or homozygous for lactation.

Results: Neither the average levels of homocysteine before and after dialysis or the reduction percentage of homocysteine varied with gender, although purification of urea was higher in women. Comparisons of homocysteine levels and percentage reduction in this ratio according to the ultra filtration of the dialyser used showed significant results. The molecular study of the gene in mutilen-tetrahydrofolate reductase enzyme showed that mutation was present in 54.8%: 45.2% with heterozygous polymorphism and 9.7% with homozygous.

Conclusions: Patients undergoing hemodialysis were found to have higher levels of urea and its clearance was greater with the higher the ratio of ultra filtration dialyser. Mutation of the gene in mutilen-tetrahydrofolate reductase enzyme was similar in our patients compared to the general population and had no impact on plasma levels of homocysteine.

Keywords: End Stage Renal Disease, Hemodialysis, Hyperhomocysteinemia, MTHFR, Uremia

Introduction

Cardiovascular disease is the primary cause of death (43.4%) in hemodialysis patients, followed by infections (14.4%) and cancer (5.4%) (1, 2). This population is affected by classical cardiovascular and other specific risk factors, such as oxidative stress

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and hyperhomocysteinemia (3, 4) which are becoming increasingly more important. Hyperhomocysteinemia is gaining ground in patients with chronic kidney disease (CKD) not only due to its relationship with kidney function but also because of its involvement in the pathogenesis of endothelial dysfunction and atherosclerosis (5, 6).

Homocysteine (Hcy) is a sulphide amino acid, an intermediary product in the metabolism of methionine. Its increase may be due to a higher production and/or a default in its metabolism, a prior alteration of the enzymes involved in its metabolism or to a deficit of host (folic acid and vitamins of group B) (7, 8). This happens frequently in CKD stage 5 patients, leading to the classic cardiovascular risk factor in this population (9). Various genetic defects have been associated with the Hcy abnormal catabolism. The most frequent mutation is the change of alanine to valine at position 677 (C677T) (10, 11). In the general population, this mutation affects around 10-15% in homozygotic polymorphism and 40-45% in heterozygotic (12).

This study, conducted in CKD stage 5-D patients, aims to define the prevalence of hyperhomocysteinemia, to assess the clearance of Hcy achieved with dialysis, and to describe the frequency of the mutation of the gene in MTHFR enzyme and its relationship with plasma Hcy levels.

Materials and Methods

Study design

The records of Hcy reduction percentage (R% Hcy), Kt/V and urea reduction percentage (R% U) were analysed twice-yearly in hemodialysis patients for seven years. The records were made in each patient with semi-annual basis. Each patient contributed with one or more until they leave the center for transfer, transplant, or death. All patients received supplements of folic acid (30-100 mg) and vitamin B6 (300mg) in a single weekly IV dose after dialysis.

All the patients were dialyzed for four hours every other day, Mondays, Wednesdays and Fridays, or Tuesdays, Thursdays and Saturdays. The dialyzers of high and low permeability in each dialysis session were used, alternating the filter if the patient had used a low permeability or high permeability. All the patients were dialyzed with high-flow dialysis with variations of Qb to 350 and 400 ml/min.

Blood records for the calculation of R% Hcy, Kt/V (using Daugirdas 2nd generation formula) and R% U were obtained in the middle of the week. Serum urea (mg/dL) and total Hcy in plasma (μmol/L) were determined in blood samples taken before (bd) and after (ad) the dialysis sessions, following the usual methodology of Kt/V of urea (13, 14). In addition, biochemical assessments were measured using standard laboratory techniques. Kt/V (Daugirdas 2nd generation), urea reduction percentage (R% U=(1-(u.ad/u.bd))×100), and Hcy reduction percentage (R% Hcy=(1-(ho.ad/ho.bd))×100) were calculated, meaning u.ad=urea after dialysis; u.bd=urea before dialysis; ho.ad=Hcy after dialysis and ho.bd=Hcy before dialysis.

The high vs. low hydraulic permeability filter (Kuf > vs. ≤20 mL/hour×mmHg) and the duration of the hemodialysis treatment were noted for each study of urea and Hcy.

The population of the sample consisted of 61 patients with CKD who were dialysed every other day, in this unit in Spain. They were not selected by random sampling procedure and their participation and inclusion in the research was determined by their presence in the dialysis centre on the dates the study was being conducted, from September 2002 to September 2009. The study was conducted with prior consent of the patient and approval by the hospital ethics committee.

Molecular study of mutation in the gene encoding the enzyme mutilated-tetrahydro-folate reductase (MTHFR):

A molecular study of the mutation of the gene encoding the mutilen-tetrahydrofolate reductase (MTHFR) enzyme was carried out in a group of patients with CKD who were dialysed every other day in this unit in Spain. The presence of the mutation C-T in position 677 of this gene (15) was studied using real time PCR with specific FRET probes for the normal and mutated variables of the gene in MTHFR enzyme to detect mutation. In this study, three types of results were obtained: absence of mutation; presence of mutation in heterozygotics; and presence of mutation in homozygotics.

Statistics

A descriptive study (mean±SD) was performed using the Student t test, and non-parametric test of Kruskal-Wallis test. The result was considered significant when p was less than 0.05.

Results

We analyzed 230 records on 61 patients (41% female) with a mean of 3.77 records per patient (range 1-8). Average year of birth was 1940±13.4. The most frequent cause of primary kidney disease was interstitial (24.6%), followed by vascular and diabetes (18% respectively) and glomerular (13.1%). The study was finally completed by 27 patients (44.3%). The drop out was due to death, transplant or transfer, 34.4%, 4.9% and 16.4% respectively. Average age at death was 66±13.3 years and the most frequent cause of death was ischemic heart disease (38.1%).

When classifying the records of Hcy bd and ad at <15 vs. ≥15 μmol/L (our laboratory establishes 15 as the normal cut-off point), it was observed that 74.3% was ≥15 μmol/L bd and 14.3% ad. The average levels of Hcy bd, ad and R% Hcy were similar in males and females. However, the clearance of urea was higher in females (Kt/V females 1.7±0.29 vs. males 1.5±0.25; R% U females 75±6.6 vs. males 70±7.0; $p< 0.05$).

Comparing Hcy levels and R% Hcy using Kuf dialyser yielded significant differences, as shown in Table 1: Hcy ad was lower and R% higher when high permeability dialysers were used. However, the levels of Hcy ad, Kt/V and R% were similar between groups.

There were no significant differences in the parameters of dialysis: dialysate flow, dialyzer membrane surface area, blood flow and session duration.

The results obtained after the molecular study of the gene in the MTHFR enzyme, conducted on 31 patients, showed that, in general, the mutation was present in 54.8%: 45.2% with heterozygotic polymorphism and 9.7% homozygotic. Accordingly, Hcy levels and R% Hcy were similar between groups, as shown in Table 2.

Table 1. Level of homocysteine according to the coefficient of system (KUF) filter: Low vs. High KUF

Homocy steine	KUF	n	mean	SD	P
Hcy Before Dialysis	Low Kuf	163	19.2	5.84	NS
	High Kuf	67	17.7	5.63	
Hcy After Dialysis	Low Kuf	163	10.9	3.92	<0.05
	High Kuf	67	8.8	3.73	
R% Hcy	Low Kuf	163	43.8	10.07	<0.05
	High Kuf	67	51.4	9.74	

SD, Standard deviation; R%, % Clearance; NS, No significante

Table 2. Levels of homocysteine as result of genetic study of the enzyme MTHFR

Homocy steine	Tyhe	n	Mean	SD	P*
Hcy Before Dialysis	No	75	19.2	6.2	
	Heterozygous	73	17.9	5.3	NS
	Homozygous	22	18.7	3.7	
Hcy After Dialysis	No	75	10.2	4.3	
	Heterozygous	73	9.72	3.5	NS
	Homozygous	22	9.55	2.6	
R% Hcy	No	75	47.68	9.9	
	Heterozygous	73	46.29	10.9	NS
	Homozygous	22	49.4	6.8	

MTHFR, Methylenetetrahydrofolate reductase; **NS,** No significant; **SD,** Standard Deviation
* **t Student test**

Discussion

Nowadays, plasma Hcy levels are being reconsidered as a cardiovascular risk factor, although future studies are needed to clarify this issue (16). However, there is no doubt that hyperhomocysteinemia exists in the chronic kidney disease population (17). In fact, our study found that hyperhomocysteinemia was present in 74% of the records predialysis, that the Hcy clearance was greater when using high Kuf dialysers and that the distribution of the mutation of the MTHFR enzyme was similar to that observed in the general population. Although the role of hyperhomocysteine as a cardiovascular risk factor in dialysis patients is well known (18, 19), studies that have reported the relationship between Hcy levels and vascular complications have so far yielded mixed results (20). Moreover, some authors find no clear association between lowered Hcy levels and a reduction in cardiovascular events in uremic patients.

In our view, high Hcy levels at the predialysis stage may produce vascular injuries of target organs, and once in dialysis high levels are not possible as Hcy is cleared by dialysis. It is possible to find situations where Hcy levels fluctuate in plasma depending on the dose of dialysis, on the dialysers used and on the administration of hosts of the MTHFR enzyme. Some dialysis patients have low Hcy levels, which are likely to be related to a poor protein-caloric intake or even to an evident malnutrition, making this factor contribute to an unclear picture (21, 22).

Ideally, dialysis should correct hyperhomocysteinaemia. In practice, this is not the case, as it persists in 14% of patients who were dialyzed. It is worth pointing out that dialytic clearance of Hcy is higher when high Kuf filters are used, whereas the clearance of urea is similar (24). This might have implications for treatment, prompting the use of these dialysers in the majority of our patients. Mutation of the gene in MTHFR enzyme was distributed in the sample population in a similar way as in the general population and it did not affect the plasma Hcy levels decisively.

In conclusion, hyperhomocysteinemia is present in a high percentage in patients with CKD stage 5D.

As its clearance is higher with the use of high Kuf dialysers, we recommend use of these filters.

Conflict of Interest

None declared.

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