The importance of homocysteine levels in the prognosis of patients with chronic renal disease and in hemodialysis patients

Importância dos níveis de homocisteína no prognóstico de pacientes com doença renal crônica e em pacientes em hemodiálise

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ABSTRACT

Introduction: Homocysteine (Hcy) is one of the metabolites of methionine (Met), an essential diet-derived amino acid. There is a close relationship between high plasma Hcy levels and declining renal function. Plasma and urinary Hcy level has been the target of studies as a biomarker that forecasts poor outcome in renal patients and in hemodialysis patients. This review evaluates the main studies that sought to correlate Hcy and poor prognosis in renal disease as well as the treatments proposed for the reduction of plasma Hcy levels in these patients. Conclusion: Hcy could be an important biomarker of renal disease progression mainly in hemodialysis patients. We emphasize the importance of normalizing plasma levels of this amino acid to ensure a better prognosis in kidney disease.

Key words: homocysteine; metabolism; chronic kidney failure.

INTRODUCTION

Homocysteine (Hcy)

Homocysteine (Hcy) is a sulfur-containing non-proteinogenic amino acid derived from the demethylation of an essential amino acid, methionine (Met). It is an intermediate amino acid in the biosynthetic pathway able to carry out Met conversion into cysteine. Met, mostly found in the liver, is converted to Hcy via S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH), which are released in numerous methylation reactions. This molecule originates from catabolism of proteins or varied dietary sources, and its metabolism occurs by two main pathways: remethylation and transsulphuration.

During methionine remethylation cycle, an enzyme action occurs both from methylytetrahydrofolate and betaine for methyl donation to this molecule, with cobalamin (vitamin B12) being one of the precursors of this activation that originates Met. On the other hand, in transsulphuration, there is an irreversible reaction in which Hcy condensates along with the serine molecule, which is catalyzed by cystathionine-β-synthase, forming cystathionine. This reaction occurs by direct action of pyridoxine cofactor (pyridoxal 5-phosphate, vitamin B6) from dietary sources.

Increased plasma Hcy positively correlates with vascular and arterial lesions, chronic kidney disease (CKD), brain lesions and skeletal system alterations, besides being considered a predictive factor of high-risk mortality.

Classification of Hcy levels in the body

According to Gascon et al. (2010), Hcy levels detectable in humans are: 5-15 µmol/l – normal; 16-30 µmol/l – moderate hyperhomocysteinemia; 31-100 µmol/l – intermediate hyperhomocysteinemia; values > 100 µmol/l – severe hyperhomocysteinemia; and 200-400 µmol/l – homocysteinuria.
Increased serum levels of Hcy are present in individuals with genetic alterations that interfere in the activities of enzymes involved in its metabolism \(^{(12, 13)}\), or even in individuals with severe nutritional deficiencies \(^{(14)}\). Homocysteinuria and hyperhomocysteinemia (HHcy) are considered independent risk factors of cardiovascular diseases, and these alterations are caused by an autosomal recessive disorder characterized by high concentrations of Hcy, both in plasma and in urine \(^{(15)}\).

In recent decades, the direct relationship between alterations in Hcy metabolism and poor prognosis of kidney diseases has been studied \(^{(7, 16)}\). Hcy and the kidney

HHcy is present in individuals with declining kidney function, so that this alteration can be considered a major factor in the progression of kidney diseases, mainly CKD \(^{(17)}\).

Hcy is known to undergo transsulphuration in the kidney. Therefore, significant alterations in enzymes and mechanisms involved in transsulphuration strongly contribute to increase Hcy levels in plasma; at the same time, there is damage to renal and cardiovascular function \(^{(17, 18)}\).

Hcy in kidney disease

Individuals with CKD or mildly decreased glomerular filtration rate (GFR) have presented high levels of Hcy \(^{(19, 20)}\).

A moderate increase in plasma levels of Hcy can be observed since the initial phases of CKD, and this increase occurs simultaneously with the decline of renal function. In fact, prevalence of altered Hcy levels in kidney disease patients can reach 84% in advanced stages of the disease \(^{(10)}\).

In contrast, Nerbass et al. (2006) \(^{(7)}\) did not find positive correlation between the increase of total Hcy levels and creatinine or serum creatinine clearance. This result makes believe that creatinine clearance is a predictive factor of renal function loss independent from total Hcy levels \(^{(7)}\).

High levels of Hcy can also decrease formation of adenosine, an important cardiac vasodilator and a constrictor of the renal vascular bed \(^{(21, 22)}\), which, besides enhancing pro-oxidizing effects, decreases nitric oxide bioavailability \(^{(23, 24)}\). Some of those actions directly affect renal function and contribute to a poor prognosis in CKD pictures.

Another important factor for the high levels of Hcy in renal patients is folate deficiency, as well as low concentrations of vitamins B\(_{12}\) and B\(_{6}\) \(^{(25, 26)}\). Therefore, the most important factor involved in pathophysiology of HHcy is the lower renal metabolism of this amino acid, followed by reduction of extrarenal metabolism (consequence of the uremic state), and the deficiency of vitamins B\(_{12}\), B\(_{6}\), and folate (indispensable to Hcy metabolism) \(^{(27, 28)}\).

The objective of the present study is to conduct a literature review, aimed at establishing a relationship between serum levels of Hcy and the presence of kidney disease, to evaluate if there is a causal and/or prognostic relationship.

METHOD

The descriptors used for the review were standardized by the controlled vocabulary Medical Subject Headings (MESH) related to Hcy and kidney disease. The used databases were Medline, Scielo and Lilacs. Just the articles with the selected headings in the title or abstract, and with abstracts available in Portuguese or English, from the beginning of the historical series until 2017, were included in this review.

Selection was made by two researchers in isolation. When there was divergence in the choice of articles, a third researcher performed analysis and made choice.

RESULTS

Using the subject headings homocysteine, hemodialysis, hemodialyses, renal dialysis, renal dialyses, chronic renal insufficiency, chronic kidney insufficiency, chronic kidney disease in the databases PubMed and Lilacs, we obtained 424 articles, of which 61 were selected. We included articles with at least two of the headings in the title or in the abstract, and that were relevant to the objective of the present study. Those that were not written in English or Portuguese were excluded.

Articles were divided into four groups according their dominant topics: 1) predictors of Hcy levels; 2) Hcy; 3) kidney disease; and 4) Hcy metabolism.

Hcy metabolism

Patients with end-stage renal disease (ESRD) have higher risk of cardiovascular events \(^{(20)}\). Several studies demonstrated that such a risk must not be attributed to just kidney injury per se \(^{(30)}\). Apparently, the high levels of plasma Hcy found in these patients are directly related to higher incidence of cardiovascular events.
As renal function decreases, Hcy levels increase, what generates endothelial dysfunction, vascular injury, intima layer proliferation and alteration of platelet aggregation and clotting. These changes directly contribute to atherosclerosis formation\(^{31-33}\). Taking these into account, diverse substances have been suggested in an attempt to minimize HHcy effects on CKD and hemodialysis patients.

**Treatments**

Perna et al. (2012)**\(^{34}\)** suggested that a significant fraction of circulating Hcy is covalently bound to albumin, what hinders its removal during dialysis. Therefore, displacement of this binding could favor availability of free Hcy for plasma clearance. The studied alternative was a treatment using N-acetylcysteine (NAC), a thiol-containing antioxidant that could fulfill this function, considering that NAC is just partially removed during dialysis**\(^{37}\)**. Treatment with NAC induced significant removal of Hcy during dialysis, and this effect was observed just during drug administration**\(^{34}\)**.

Ventura et al. (1999)**\(^{36}\)** showed that one single intravenous (i.v.) dose of NAC reduced Hcy in the plasma of healthy individuals mediated by increased urinary excretion of thiol. Scholze et al. (2004)**\(^{37}\)** reported that i.v. administration of NAC (5 g in glucose solution 5% for 4 hours) in renal patients, during a hemodialysis session, was able to normalize total concentrations of Hcy at the end of the session. Thaha et al. (2006)**\(^{38}\)**, in a similar evaluation, obtained the same results.

Oral administration of NAC reduces cardiovascular risk, but is less effective than i.v. administration. This substance is safe to contribute to HHcy normalization in patients with CKD undergoing hemodialysis**\(^{35,39-41}\)**, although its effect is limited to time of administration.

Scholze et al. (2004)\(^{37}\), in a crossover randomized study, evaluated 20 patients with end-stage renal failure, to investigate metabolic and hemodynamic effects in the i.v. administration of NAC during a hemodialysis session. The authors verified a positive correlation between increased removal of Hcy and increased dose of NAC during the session. Plasma concentration of Hcy prior to the hemodialysis session was 58 ± 22%, while in the presence of NAC, plasma concentration of Hcy was significantly reduced to 12 ± 7%.

These effects suggest that i.v. administration of NAC can reduce mortality by cardiovascular causes in patients with end-stage renal failure, and consequently, can be a novel and promising therapeutic approach to reduce atherosclerosis in hemodialysis patients**\(^{37}\)**.

Reduction of total values of plasma Hcy during supplementation of high doses of folic acid, vitamin B\(_6\) and B\(_{12}\) was also demonstrated. Reduction in plasma concentration of Hcy with this vitamin supplementation is 10%, in this study this reduction was suggested to have contributed to decrease cardiovascular risk in these patients, as well as mortality rates**\(^{42}\)**.

Tremblay et al. (2000)**\(^{14}\)** conducted a study to evaluate the effects of supplementation with a hydrosoluble multivitamin formulation (DiaVite\(^{®}\)) containing folic acid, vitamin B\(_6\) and vitamin B\(_{12}\). This supplement was chronically administered in CKD patients in advanced stage and undergoing hemodialysis. The evaluation suggests that an enteral formulation of the hydrosoluble multivitamin DiaVite\(^{®}\) is ideal for patients with end-stage kidney failure treated by hemodialysis. The authors also point that DiaVite\(^{®}\) presents high efficiency to normalize concentrations of complex B vitamins. Its administration causes very significant reductions in Hcy serum levels, what in the long term can be beneficial as a preventive measure. They also suggested that supraphysiologic doses of folic acid could be especially useful to lower HHcy. Treatment with parenteral DiaVite\(^{®}\) was classified by the authors as a safe and low-cost option**\(^{43}\)**.

Elian and Roffer (2002)**\(^{40}\)** investigated the importance of folic acid supplementation in patients with CKD and verified normalization of plasma total Hcy concentration and the reflex of this treatment on concentrations of vitamin B\(_{12}\). Patients were treated with folic acid (5-6 mg/day), pyridoxine (5-10 mg/day), and vitamin B\(_{12}\) (6-10 mg/day) orally, and the values of Hcy and methylmalonic acid (MMA) were measured eight and 16 weeks after treatment beginning. Vitamin B\(_{12}\) supplementation was able to reduce plasma Hcy levels**\(^{40}\)** by 25%. These authors found partial efficacy of folic acid supplementation to correct Hcy increase during hemodialysis in CKD patients. This is due to a deficiency in folic acid metabolism, to availability of 5-methyltetrahydrofolate (MTHF) and to transmembrane transport of MTHF**\(^{40}\)** in these patients.

Methylation is known to cause the repair of membrane proteins that were damaged, for example, by an oxidation process. On the other hand, methylation reduction is responsible for accumulation of altered proteins. MTHF is the reduced circulating form of folate that is capable of methylation, besides being efficient to reduce plasma Hcy**\(^{45}\)**. According to the authors, the oral dose of 15 mg/day of MTHF during two months decreases by 70% Hcy levels in patients on hemodialysis, with five patients reaching the highest level of normality in the study**\(^{45}\)**.

Conversely, an experimental study in rats showed that intestinal absorption of MTHF is impaired in CKD**\(^{46}\)**. Thus, to understand all the effects of MTHF treatment in hemodialysis, further studies are necessary to evaluate safety of this supplementation in the long term and if there are cardiovascular benefits added to these effects.
Another treatment considered for HHcy is acid folic supplementation. Thambyrajah et al. (2000)\(^{(47)}\) verified that supplementation failed in normalization of Hcy levels in predialysis patients, and did not act in endothelial dysfunction, what suggests that monotherapy with folic acid is not efficient on vascular disease of uremic patients and those with HHcy\(^{(47)}\).

Approaches non-related to vitamin supplementation were also studied. When taurine thiol was used with mesna, this association was verified to be involved in reduction of Hcy levels during hemodialysis. Mesna caused rapid sustained Hcy decrease; this substance was able to enhance Hcy dialytic clearance\(^{(49)}\).

Taes et al. (2004)\(^{(48)}\) tested inhibition of endogenous methylation with supplementation of creatine during four weeks aimed at decreasing Hcy concentrations in hemodialysis patients. Supplementation with creatine (2 g/day) did not reduce Hcy concentrations in patients that had already received previous treatment with folic acid, vitamins B\(_6\) and B\(_12\). In this case, creatine just presented effects upon patients’ muscular performance and quality of life\(^{(49)}\).

Perna et al. (2007)\(^{(50)}\) studied exogenous supplementation of L-propionyl carnitine, a derivate of trimethyllysine that, after catalyzed by S-adenosylmethionine methyltransferase, produces Hcy precursors. The study aimed to determine if that exogenous administration would affect plasma levels of Hcy in patients with end-stage CKD on hemodialysis. Treatment did not significantly influence Hcy levels, demonstrating that this supplementation does not directly impact the mechanisms of Hcy metabolism, specifically in patients with ESRD.

**Predictors of Hcy levels**

A cross-sectional study searched to correlate Hcy levels with those of serum creatinine in patients with creatinine levels of 1.5-8 mg/dl aged 18-60 years. In this evaluation, the authors found a moderate correlation between age and creatinine clearance with Hcy levels in pre-hemodialysis patients with CKD. The decrease of 1 ml/minute in creatinine clearance brought an increase of 0.2 mmol/l in Hcy levels; the increase of one year in age raises 0.2 mmol/l Hcy levels\(^{(51)}\).

Regarding participation of oxidative stress and activation of pro-oxidant enzymes in HHcy, in vitro studies demonstrated that redox reactions in the thiol group affect the functions of endothelial cells. Strong association was found between homocysteinemia and plasma levels of thiol in patients with end-stage renal failure, what suggests that redox status alterations in the thiol group can be a possible pathogenic mechanism underlying HHcy in cardiovascular toxicity present in these patients\(^{(52)}\).

Dimethylglycine (DMG) was questioned to be related with HHcy by inhibition of betaine-homocysteine S-methyltransferase (BHMT). BHMT is a zinc metalloenzyme that catalyzes the transfer of a methyl group from betaine to Hcy, converting glycine betaine to DMG. DMG accumulation would lead to HHcy by BHMT inhibition. Reduction of BHMT activity is important in the pathogenesis of HHcy in CKD, and zinc deficiency can contribute to this reduction\(^{(53)}\).

Knowledge on the role of vitamins of the B complex, except vitamins B\(_6\) and B\(_12\), which have already been mentioned, as possible determinants of HHcy in renal failure, is still limited. What is known is that vitamin B\(_6\) (thiamine pyrophosphate) and vitamin B\(_2\) (riboflavin) are involved in the metabolism of Met and Hcy. Bioavailability of thiamine or riboflavin after fasting is believed to be a predictor of Hcy plasma levels in patients with ESRD.

One of the research groups that tested this hypothesis conducted a cross-sectional study in patients non-supplemented with vitamins that were kept in continuous outpatient peritoneal dialysis. Bioavailability of red blood cells, thiamine and riboflavin, along with other important predictors of Hcy plasma levels, was considered in the assessment. The study results showed that riboflavin bioavailability is, actually, a predictor of Hcy plasma levels in patients on peritoneal dialysis\(^{(54)}\).

Bayes et al. (2003)\(^{(55)}\) suggested a hypothesis opposite to the previously presented data, whereby the main risk factors for mortality in hemodialysis patients would not be only HHcy, but also lipid peroxidation and inflammatory state. Patients evaluated in that study received vitamin supplementation (complex B and folic acid) during the hemodialysis period and there was no correlation between Hcy plasma levels and the different mortality causes. Thus, one can suggest that other deleterious pathways, also active in end-stage CKD, contribute to mortality in hemodialysis patients\(^{(55)}\). Apparently, just the blockage of HHcy is not enough to ensure a better prognosis for these patients. Other evaluations are necessary with a larger number of patients to support this hypothesis.

**Genetics**

Wrone et al. (2001)\(^{(56)}\) studied if total plasma Hcy is associated with genetic alterations, specifically the C677T mutation of the methylenetetrahydrofolate reductase (MTHFR) gene, and if this association would be involved with the highest incidence of cardiovascular diseases in CKD patients. To that intent, they studied a cross-sectional sample of 459 patients with end-stage renal failure on hemodialysis. Genotype C677T of MTHFR is directly related to cardiovascular diseases in CKD, being a potential marker of abnormal Hcy metabolism in CKD.
C677T mutation of MTHFR gene, located on chromosome 21, is considered a genetic risk factor for vascular disease (57). A body of evidence described the influence of C677T genotype in Hcy metabolism, including in individuals with low folate level (58-61). However, studies on the gene mutations that can be related to HHcy should be expanded to verify other possible alterations in the pathways of this amino acid synthesis.

CONCLUSION

The studied works showed the main causes of increased plasma Hcy in CKD, which range from vitamin or enzyme deficiency to alterations in genes related to the enzymatic pathway and to cofactors involved in its metabolism. However, the exact mechanisms involved in HHcy have not yet been fully elucidated.

RESUMO

Introdução: A homocisteína (Hcy) é um dos metabólitos da metionina (Met), um aminoácido essencial proveniente da dieta. Existe uma estreita relação entre os altos níveis plasmáticos de Hcy e o declínio da função renal. A Hcy plasmática e urinária tem sido alvo de estudos como um biomarcador capaz de sinalizar o prognóstico em doentes renais e em pacientes em hemodiálise. Esta revisão avalia os principais estudos que buscarem correlacionar a Hcy e o prognóstico da doença renal e descreve os tratamentos propostos para a redução dos níveis plasmáticos de Hcy nesses pacientes. Conclusão: A Hcy pode ser um biomarcador da progressão na doença renal, principalmente em pacientes hemodiálíticos. Ressaltamos a importância da normalização dos níveis plasmáticos desse aminoácido para garantir um melhor prognóstico na doença renal.

Unitermos: homocisteína; metabolismo; falência renal crônica.


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