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Association of Serum Homocysteine with Anemia in Maintenance Hemodialysis Patients

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Abstract: To investigate the relationship between homocysteine level and anemia in maintenance hemodialysis patients, a cross-sectional study that was conducted on patients with end-stage renal disease (ESRD) undergoing maintenance hemodialysis treatment. The study was carried out on 39 (F=15 M=24) stable hemodialysis HD patients. Mean ages of patients were 46±18 years. The length of the time patients had been on hemodialysis was 30±35 months (median: 18 months). The value of serum homocysteine of all patients was 5±2 µmol/L (median : 4.5 µmol/L). Mean±SD of hemoglobin and hematocrit level of all patients were 9±2 g/dl (median : 9 g/dl), and 28±6% median: 29% respectively. In this study, we assessed for the first time the positive relation of serum homocysteine with anemia in patients on maintenance hemodialysis. In addition, in male HD patients a significant positive correlation of serum homocysteine with serum ferritin and a significant inverse correlation of serum homocysteine with serum total iron binding capacity (TIBC) were seen. It is possible that the high levels of serum homocysteine in some conditions may increases the micro inflammatory state of uremia in hemodialysis patients and had a role in intensification of anemia. In the meantime more research needs to confirm our conclusion.

Key words: Hemodialysis, homocysteine, end-stage renal failure, anemia, ferritin

Introduction

Anemia is a consistent finding in chronic renal disease, affecting up to 90% of patients, and the central role of anaemia in the development of cardiovascular dysfunction is now well established (Nasri, 2003; Parfrey, 2001). Homocysteine (Hcy) is a sulphur amino acid formed from methionine during transmethylation, and is either salvaged to methionine by a folate- and cobalamin-dependent re-methylation reaction or directed toward degradation by the vitamin B6-dependent enzyme cystathionine ß-synthase (Moudd *et al*., 1989). Large studies have demonstrated that moderate hyperhomocysteinaemia is an independent risk factor for premature atherosclerosis and cardiovascular disease (Eikelbloom *et al*., 1999). Mild-to-moderate elevations in plasma total homocysteine (tHcy) levels are observed in the great majority (>85%) of patients with end-stage renal disease who are undergoing maintenance dialysis (Foley *et al*., 1998). In maintenance hemodialysis (MHD) patients, the association between tHcy and clinical outcome is inconsistent and even paradoxical. Some studies have shown a poor outcome in MHD patients with hyperhomocysteinemia. Recent studies have suggested that a decreased, not an increased, tHcy concentration is related to a higher prevalence of cardiovascular disease and poor outcome in these individuals (Wrone *et al*., 2001; Suliman *et al*., 2000). Indeed plasma levels of total homocysteine are influenced by nutritional status in patients with chronic kidney disease (Suliman *et al*., 2004; Mallamaci *et al*., 2002). It is believed that both inflammation and protein-energy malnutrition, each independently or together as the "malnutrition-inflammation cachexia syndrome" (MICS) are responsible for this condition (Kalantar-Zadeh *et al*., 2003). There is quiet little information about the relationship between homocysteine level and anemia in maintenance hemodialysis patients. We hypothesed that there may be an association between serum homocysteine level as a marker of nutritional status and the intensity of anemia existed. We therefore test the association of anemia and serum homocysteine in a group of end-stage renal failure patients undergoing regular hemodialysis.

Materials and Methods

This is a cross-sectional study that was conducted on patients with end-stage renal disease (ESRD) undergoing maintenance hemodialysis treatment with acetate basis dialysate and polysulfone membranes. Exclusion criteria for patients were using of angiotensin converting inhibitors ,angiotensin receptor antagonists, and aluminum hydroxide jells as well as active or chronic infection before the study. Serum homocysteine (total) was measured as follows. Blood samples were drawn after an overnight fast. Each blood samples were

Table 1: Mean±SD, Minimum and Maximum of age, duration and dosage hemodialysis and also laboratory results of all hemodialysis patients

Total patients n=39		Mean±SD	Median
Age	years	18±46	42
DH*	months	35±30	18
Dialysis dose	sessions	381±279	156
URR	%	8±58	58
HCO_3	mEq/L	20±2.3	20
iPTH	Pg/ml	434±455	309
Ca	mg/dl	7.7±1	8
P	mg/dl	6.4±1.9	6.4
Alp	IU/L	533±890	444
BMI	kg/m	21.6±4.3	21
Alb	g/dl	0.5±3.8	4
Homocysteine	µmol/L	5±2	4.9
Ferritin	ng/dl	519±299	426
Iron	µg/dl	350±454	69
TIBC	µg/dl	968±562	1059
CRP	µg/l	6.7±8.8	6
mg/dl	Chol	38±116	110
Hgb	g/dl	2±8.9	9
Hct %		6±28	29

*duration of dialysis

centrifuged within 15 min of venepuncture, and were measured by enzyme-linked immunosorbent assay (ELISA) method using DRG kits of Germany .Serum total Homocysteine (Hcy) have a normal range of 25-125 µmol/L. Also peripheral venous blood samples were collected for biochemical analysis including serum post and predialysis blood urea nitrogen (BUN), Chol, albumin (Alb), C-reactive protein (CRP) were measured using standard methods. Intact serum PTH (iPTH) was measured by the radioimmunoassay (RIA) method using DSL-8000 kits of USA (normal range of values is 10-65 pg/ml). Plasma HCO_3 and blood PH was measured by arterial blood gas. Levels of serum iron, total iron binding capacity (TIBC) and serum ferritin (by RIA method) were measured using standard kits. For patients also complete blood count containing hemoglobin (Hgb) and hematocrit (Hct) were measured using Sysmex-KX-21N Cell counter. For the efficacy of hemodialysis the urea reduction rate (URR) was calculated from pre- and post-blood urea nitrogen (BUN) data. Body mass index (BMI) calculated using the standard formula (postdialyzed weight in kilograms/height in square meters; kg/m^2). Duration and doses of hemodialysis treatment were calculated from patients' records. The duration of each hemodialysis session was four hours. For statistical analysis, the data are expressed as the Mean ± SD and madian values. Statistical correlations were assessed using the partial correlation test. Statistical analysis was performed on total hemodialysis (HD), females, males, diabetics and non diabetics populations separately. All statistical analyses were performed using SPSS (version 11.5.00). Statistical significance was determined at a p-value <0.05.

Results

The study was carried out on 39 (F=15 M=24) stable hemodialysis (HD) (diabetic = 12 non-diabetics = 27) patients. Table 1 shows patients' data. Mean ages of patients were 46±18 years. The length of the time patients had been on hemodialysis was 30±35 months (median: 18 months). The value of serum homocysteine of all patients was 5±2 µmol/L (median:4.5 µmol/L). The value of serum homocysteine in the female and male groups were 5±3 µmol/L (median :3.7 µmol/L) and 5±2 µmol/L (median :4.9 µmol/L) respectively. Mean±SD of hemoglobin and hematocrit level of all patients were 9±2 g/dl (median : 9 g/dl), and 28±6% (median: 29 %) respectively. In all patients a significant positive correlation of serum homocysteine with hemoglobin level ($r = 0.37$, $p = 0.041$; Fig. 1) and a significant positive correlation of serum homocysteine with hematocrit level ($r = 0.37$, $p = 0.045$; Fig. 2) (adjusted for age, duration and doses of dialysis, DM, URR and plasma HCO_3, serum CRP, P and Ca for two correlations) were seen. In male HD patients a significant positive correlation of serum homocysteine with serum ferritin ($r = 0.59$, $p = 0.034$; Fig. 3) and a significant inverse correlation of serum homocysteine with serum total iron binding capacity (TIBC) ($r = - 0.52$, $p = 0.048$; Fig. 4) (adjusted for age, duration and doses of dialysis, BMI, URR, HCT and plasma HCO_3, serum CRP, Chol, P, iPTH and Ca for two correlations) were found.

Discussion

In this study, we assessed for the first time the positive relation of serum homocysteine with anemia in patients on maintenance hemodialysis. In addition, in male HD patients a significant positive correlation of serum homocysteine with serum ferritin and a significant inverse correlation of serum homocysteine with serum total iron binding capacity (TIBC) were seen. The mechanism by which homocysteine exerts its effects has not been clearly defined, although it is generally accepted that the accumulation of homocysteine in plasma can damage the endothelium. It has been suggested that homocysteine may induce vascular injury (including endothelial dysfunction, smooth muscle cells proliferation and thiolation of lipoprotein) and affect platelet aggregation and coagulation (Jakubowski, 1997). In contrast to our findings in the study of Carluccio *et al.* (2002) homocysteine serum levels were measured in patients with end-stage renal disease in relation to severity of renal anemia and oxidative stress parameters such as 4-hydroxynonenal (HNE) and malondialdehyde (MDA). The predialytic homocysteine serum levels of the patients are five times as high as in healthy controls. They found that homocysteine does not correlate to

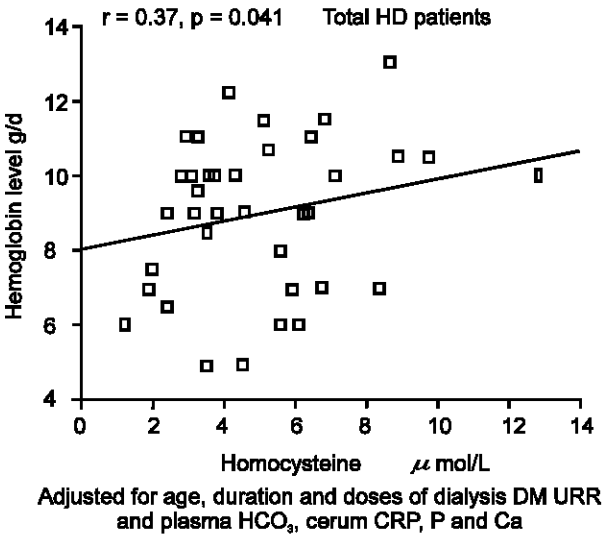

Fig. 1: Significant positive correlation of serum homocysteine with hemoglobin level

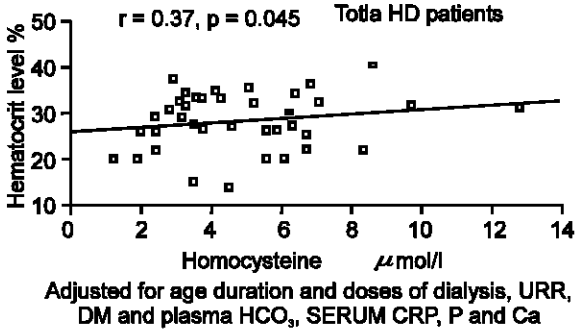

Fig. 2: Significant positive correlation of serum homocysteine with hematocrite level

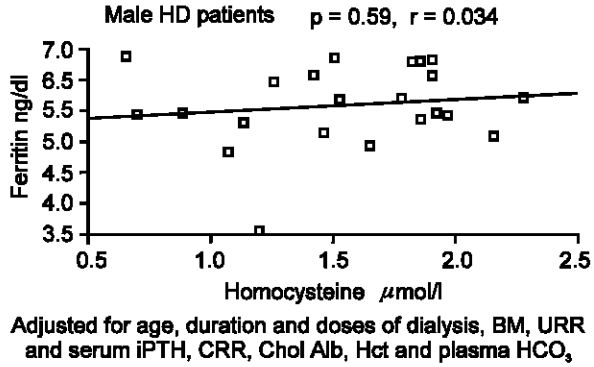

Fig. 3: Significant positive correlation of serum homocysteine with serum ferritin

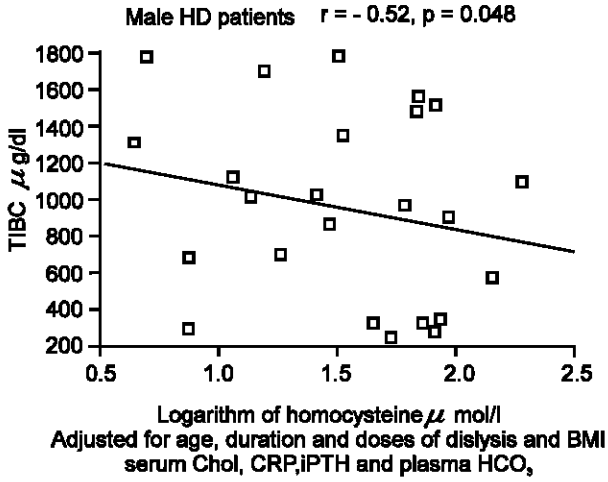

Fig. 4: Significant inverse correlation of serum homocysteine with serum total iron binding capacity (TIBC)

hemoglobin concentration and to oxidative stress (Carluccio *et al.*, 2002). It was shown that high levels of serum ferritin are engendered by inflammation independently of iron stores (Kalantar-Zadeh *et al.*, 2003) and serum ferritin is also an acute phase reactant (Kalender *et al.*, 2002; Kalantar-Zadeh *et al.*, 2001; Rogers, 1996). Indeed anemia of end-stage renal disease can be managed relatively successfully by recombinant human erythropoietin. Iron administration plays a central role in enhancing anaemia responsiveness to EPO. Serum ferritin concentration is a commonly used marker of iron status in maintenance dialysis patients (Kalantar-Zadeh *et al.*, 1995). It was shown that a low serum ferritin concentration is a reliable indicator of iron deficiency among ESRD patients. However, a high serum ferritin may not be an optimal indicator of "increased" iron stores among dialysis patients because it is an acute-phase reactant and its increase in dialysis patients may be based on the factor unrelated to iron stores such as inflammation (Kalantar-Zadeh *et al.*, 2003). While MICS may play a central role in poor clinical outcome including a high rate of mortality and hospitalization and diminished quality of life, it may also lead to hyperferritinaemia and refractory anaemia including EPO hyporesponsiveness in these individuals (Kalantar-Zadeh *et al.*, 2003; Trey and Kushner, 1995). Indeed the erythropoiesis-suppressing effect of inflammation is mainly due to increased activity of the proinflammatory cytokines (Wrone *et al.*, 2001; Suliman *et al.*, 2004). *In vivo*, the cytokines act in concert to affect precursor cells at different stages of erythropoiesis. Cytokines, TNF-alfa and IL-1 have been extensively studied (Trey and Kushner, 1995; Means, 1999). Hence it is possible that high levels of serum homocysteine in some conditions increase the micro inflammatory state of uremia in hemodialysis patients and had a role in intensification of anemia. In the meantime more research needs to confirm our conclusion.

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