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Serum Folate and Vitamin B12 Levels in Hemodialysis Patients: Is There any Correlation with Plasma Homocysteine Levels?

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Introduction: Deficiencies of water soluble vitamins such as folate and vitamin B12 has been reported as etiologic factors of hyperhomocysteinemia. This study was conducted to find whether there is a correlation between serum levels of these vitamins and plasma total homocysteine (tHcy) levels.

Material and Methods: 19 hemodialysis subjects were enrolled. The study group comprised 52.6% girls and 47.4% boys aged 80-324 (204.7±78.4) months who were on dialysis from 1.5-153 (42.1±43.3) months ago. All patients were supplemented by folate and 15 cases were received oral vitamin B12. Folate serum levels <1.5 ng/ml were defined as low (deficiency). As for vitamin B12, levels < 120 pg/ml, 120-160 pg/ml were defined as deficient and borderline, respectively. Plasma Hcy levels of 5-15 µmol/L and > 15 µmol/L were defined as normal and hyperhomocysteinemia, respectively. The correlation between the serum levels of vitamins and plasma Hcy levels was checked by the Pearson correlation test and P-values <0.05 and r>0.7 indicated a good (significant) correlation.

Results: 13 patients (68.4%) had hyperhomocysteinemia whereas plasma tHcy levels were normal in 6 (31.6%). No patient had folate or vitamin B12 deficiency. There was no correlation between tHcy levels and serum vitamin B12 (P=0.621, r=1) and serum folate levels (P=0.571, r=1).

Conclusions: Normal and even high serum levels of folate and vitamin B12 cannot prevent the occurrence of hyperhomocysteinemia in hemodialysis patients.

Keywords: Hemodialysis; Folate; Vitamin B12; Homocysteine; Hyperhomocysteinemia.

Running Title: Serum Folate and Vitamin B12 Levels in Hemodialysis

Introduction

Hyperhomocysteinemia is an important independent cardiovascular risk factor [1]. This metabolic abnormality is present in 80-100% of hemodialysis (HD) patients [2,3], which do not usually normalize by dialysis [4,5].

Homocysteine (Hcy) is a nonessential sulfur-containing amino acid normally produced by demethylation of methionine. The active forms of folate and vitamin B12 play a role as a cofactor in the metabolic pathways of Hcy [6-8]. The risk of the deficiency of water soluble vitamins in dialysis

patients is high [9,10]; therefore, supplementation with these vitamins is recommended [11]. Plasma total Hcy (tHcy) levels of 15-30, 30-100 and >100 µ mol/L are considered mild, moderate, and severe hyperhomocysteinemia [12].

The objectives of this study were to determine whether serum vitamin B12 and folate are normal in hemodialysis (HD) subjects who receive vitamin supplements and to evaluate whether there is a correlation between serum levels of these vitamins and plasma tHcy levels.

Materials and Methods

In this cross-sectional observational study, 19 HD subjects were enrolled. The study group comprised 10 (52.6%) girls and 9 (47.4%) boys aged 80-324 (204.7±78.4) months [6.7- 27 years] who were on dialysis from 1.5-153 (42.1±43.3) months ago. Patients were treated by bicarbonate hemodialysis 8-12 (10.2) hours weekly in 2 or 3 divided sessions. Dialysis was performed with Fresenius 4008 (Germany), and AK95 and AK96 (Swiss) machines. High flux synthetic membranes (PES 130) were used in children >40 kg, while in patients <40 kg low flux membranes (R3-R5 and Poly Sulfone membranes) were applied. The blood flow rates were regulated on 100-300 cc/min**.** The dialysis dose was determined by single pool kinetics (sp Kt/V). Lower doses of dialysis were recommended in some patients because they did not agree to have more dialysis sessions.

All patients were supplemented by folate and low dose oral vitamin B12 before or immediately after receiving dialysis. The majority of our cases were supplemented with nephrovite tablets containing 0.5 mg folate and 6 µg vitamin B12. Some patients received extra folate as folic acid tablets. The dose of folate supplementation was 1-10 (5±2.1) mg/day and 15 cases received low dose oral vitamin B12 (as nephrovite or B complex tablet) 3-6 (5.8 ±0,72)µg/day. Fasting blood sampling for measuring the plasma homocysteine levels was done during routine monthly sampling.

Laboratory tests used in the study

Serum vitamin B12 and folate levels were checked by the electrochemiluminescence immunoassay (ECLIA) method on Elecsys and Cobase e Immunoassay Analyzer (Roche Cobase e 411 system). The sensitivity of the tests for vitamin B12 and folate was 30 pg/ml and <20 ng/ml, respectively. Folate serum levels >20 ng/ml were only reported as high. Plasma Hcy levels were measured by the Axis –Shield kit. Serum folate levels of 1.5-17 ng/ml were considered normal, and levels <1.5 ng/ml and > 17 were defined as low (deficiency) and high, respectively. As for vitamin B12, levels < 120 pg/ml, 120-160 pg/ml, 160-970 pg/ml, and >970 pg/ml were defined as deficient, borderline, normal, and high, respectively. Plasma Hcy levels of 5-15 µmol/L and > 15 µmol/L were defined as normal and hyperhomocysteinemia, respectively.

Statistical analysis

Descriptive statistics included mean ± SD for continuous data and percentage for categorical data. Chi square and independent T tests were used for data analysis. Univariate analysis was performed using a model with Hcy, serum folate and vitamin B12 as dependent variables, and dialysis duration, age, sex, hours of dialysis per week, and dosage of folate and vitamin B12 supplement as independent variables. The normality of the variables was checked by one sample Kolmogorov-Smirnov test. Independent T and Mann-Whitney tests were used for analysis of variables with normal (vitamin B12) and abnormal (folate) distribution. P-values <0.05 were considered significant. The correlation between the serum levels of vitamins and plasma Hcy levels was checked by the Pearson correlation test and P-values <0.05 and r>0.7 indicated a good (significant) correlation. Written consent was obtained from patients or their parents. The study was funded by a research grant from Mashhad University of Medical Sciences and the study was approved by the local ethic committee.

Results

The plasma tHcy level was 7-30 (18.3±5.8) µmol/L; 13 patients (68.4%) had hyperhomocysteinemia whereas plasma tHcy levels were normal in 6 (31.6%). The lowest serum folate level was 8.15 ng/ml and no cases of folate deficiency were observed. Serum folate levels were high in 13 (68.4%) patients. Serum vitamin B12 levels were 380-1730 (873± 370) pg/ml and no patient had vitamin B 12 deficiency. Vitamin B12 levels were high in 7 patients (36.8 %). The dose of supplementary folate in patients with hyperhomocysteinemia and those with normal tHcy plasma levels was 1-10 (4.7) mg/day and 2.5-5 (4.5) mg/day, respectively. The dose of vitamin B12 supplement in these two groups was 0-6 (5.5) µg/day and 0-6 (4.5) µg/day, respectively. There was no significant difference in the mean serum vitamin B12 level between those with normal and

high plasma tHcy levels (P=0.607). Patients with hyperhomocysteinemia were significantly older than those with normal plasma homocysteine levels (P=0.01) (table 1).

Independent sample T and chi square tests were used to compare serum vitamin B12 levels among different groups of patients. There was no significant difference in age, duration of dialysis, hours of dialysis per week, dosage of supplementary vitamin B12 and serum levels of vitamin B12 (P>0.05 for all), whereas serum vitamin B12 levels were significantly higher in females than males (P=0.025) (table 2).

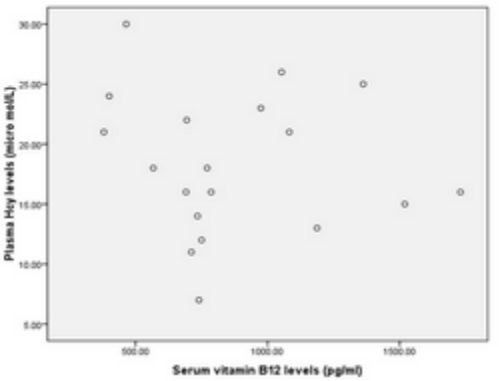

Figure 1. Correlation between serum vitamin B12 concentration and plasma homocysteine levels ⇧

Table 1. Plasma Hcy and serum folate levels in patients with different supplementary folate doses ⇧

Variable	Case supplemented by folate ≥1mg/day (mean ±SD)[2]	Case supplemented by folate <1mg/day (mean ±SD)[2]	P value
[1] Plasma Hcy concentration (μ mol/L)	16.35±4.72	16.55±6.17	**0.928**
Serum folate concentration [1] (ng/ml)	18.97±3.75	18.32±4.75	**0.707**
Total number (%)	6(31.7)	13(68.4)	

1) dependent variables 2) Independent variables
Independent T was used for analysis of variable

Table 2. Characteristics of the patients, and mean ± standard deviation of clinical and biochemical data in cases with hyperhomocysteinemia versus those with normal homocysteine levels. ⇧

	Patients with normal plasma tHcy (5-15 (μmol/L)	Patients with hyperhomocysteinemia plasma tHcy>15 μmol/L	P value
Age (month)[1]	126±41.83	241±63.03	0.001
Male **Female**	3(15.8%) 3(15.8%)	6(31.6%) 7(36.8%)	0.876
Dialysis duration (month)[1]	18.3±14.5	53.11±48,13	0.105
Hours of dialysis per week[1]	9.4±1,5	10.6±1.8	0.181
Serum folate levels (ng/ml)[1]	19.9±2.6	17.4±5,17	0.278
Serum vitamin B12 levels (pg/ml)[1]	904±336.7	803±393.5	0.607

Values are presented as number (%) or mean standard deviation.1: independent t-test, 2: Chi square tes

There was no significant difference in the mean doses of supplementary vitamin B12 between males and females (P=1). The correlation between mean ± SD serum vitamin B12 and folate levels with tHcy plasma levels was analyzed using Pearson correlation test. There was no correlation between tHcy levels and serum vitamin B12 (P=0.621, r=1) (Fig. 1) and serum folate levels (P=0.571, r=1).

Discussion

It is believed that hyperhomocysteinemia in HD patients is a metabolic impairment rather than diminished excretion of this amino acid [13-14]. Homocysteine is the transmethylation product of methionine. Two different remethylation pathways exist. The first pathway requires reduced cobalamin levels as a cofactor and 5-ethyltetrahydrofolate as a methyl donor produced by a reaction catalyzed by 5, 10-ethylenetetra hydrofolate reductase [15]. Decreased

remethylation of methionine results in hyperhomocysteinemia.

Although the reference intervals of cobalamin and folate were defined in healthy population, a value above the lower reference limit may not exclude a deficiency state. Two new markers, plasma Hcy and methylmalonic acid (MMA), are purposed as the markers of functional status of cobalamin and folate in the tissue [7]. Elevated plasma Hcy concentrations have been reported in patients with cobalamin or folate deficiency [16, 17].

Plasma homocysteine is a very sensitive marker of folate and vitamin B12 status and is inversely related with the plasma levels of these substances. The increase in homocysteine level occurs long before classic deficiency of folate and vitamin B12 becomes evident [18]. Folate and cobalamin statuses are important modifiable determinants of plasma total Hcy in the general population, and a negative correlation is observed between plasma total Hcy concentration and these vitamins even within their established normal and subnormal concentration ranges [19]. Considering the fact that a serum concentration more that the lower reference limit for folate and vitamin B12 may not exclude the deficiency of these vitamins and plasma Hcy is a very sensitive marker of folate and vitamin B12 status, functional folate and vitamin B12 deficiency determined by high plasma Hcy concentrations was a prevalent finding of our series. Our study supported the literature that purposed serum levels of folate and vitamin B12 as non sensitive markers of plasma Hcy concentration. Indicators of folate and vitamin B12 status are divided into two main types of static (direct vitamin assays) and functional indices (mainly metabolite assays). Serum folate and vitamin B12 and red blood cell folate, as well as serum holo transcobalamin II, a newer indicator of vitamin B12 status, represent the main static indicators while serum methylmalonic acid (MMA) and Hcy are the most common functional indices used to assess folate and vitamin B12 status. The main limitation of our study was that we only evaluated one static index of folate and vitamin B12 status, i.e. the serum concentrations of these water soluble vitamins, without assessing the folate concentration in red blood cell as another static index of folate status and serum holo transcobalamin II as an static index of vitamin B12 status.

Elevated Hcy with normal MMA suggests folate insufficiency. Elevated concentrations of both metabolites strongly suggest vitamin B12 insufficiency although concomitant folate insufficiency also can mimic this abnormal finding [20]. As we did not measure the serum level of MMA in our cases, we cannot define whether patients with high plasma Hcy concentration had functional deficiency of folate or vitamin B12 or both.

Although the majority of the studies have reported a positive relationship between plasma Hcy and folate and vitamin B12 status, a few investigations have revealed that both MMA and homocysteine can be elevated in the absence of either folate and/or vitamin B12 insufficiency, thereby limiting their specificity [20].

It is believed that for determining the effect of nutritional intervention, serum folate, vitamin B12, and red cell folate concentration are the principal indicators. Serum folate is a less reliable indicator than red cell folate which provides a more stable reflection of chronic folate status [20]. However, the measurement of red cell folate is a more reliable method for folate status; it has proven to be the most problematic and different factors affect the final red cell folate concentration measurement.

Evaluation of 296 cases with a diagnosis of mental disease revealed that 35-40% of the patients with low serum cobalamin or low blood folate exhibited normal values of plasma Hcy. These patients possibly had normal levels of vitamins in the tissue. It was interesting that 7.5% of the patients with normal serum cobalamin levels had increased levels of plasma Hcy, indicating tissue cobalamin deficiency despite normal serum cobalamin levels [21]. In our cases, despite normal or high serum levels of folate and vitamin B12, approximately 70% of the subjects had high plasma Hcy concentrations. An extended study in Europe reported plasma folate and cobalamin as strong predictors of total plasma Hcy [22].

In contrast to our findings, an inverse association has been reported between blood folic acid and Hcy concentration in a number of studies [23-26]. These studies have been conducted in different groups of normal population including the women of childbearing age [26], elderly population [23], middle aged adults [24], and diabetic patients with retinopathy [25]. Fotiou et al [25] detected an inverse association between vitamin B12 and Hcy levels in diabetic retinopathy patients.

Evidence shows that elevated plasma total Hcy is linked both to an inadequate status of vitamin cofactors including folate, vitamin B12, and B6, and to genetic defects in enzymes involved in Hcy metabolism [27]. Patients with low circulating folate or vitamin B12 concentrations have higher fasting total Hcy concentrations [24,28].

The Tehran Homocysteine Survey (2003–2004), a cross – sectional population based study by Fakhrzadeh et al [29], revealed that folate and vitamin B12 deficiency were prevalent in healthy Iranian adults. Folate deficiency, low serum vitamin B12 levels, and hyperhomocysteinemia were found in 97-98%, 26-27%, and 41-73% of the participants, respectively. Hyperhomocysteinemia was found in 73% of men and 41% of women. They also found an inverse correlation between log total Hcy and serum folate and vitamin B12. In our subjects who were hemodialysis children and young adults, the prevalence of hyperhomocysteinemia in boys and girls was 76.5% and 70%, respectively. It means that the prevalence of hyperhomocysteinemia in dialysis boys was similar to normal Iranian adults, while hyperhomocysteinemia was more common in dialysis girls compared with normal adult women (the ratio of hyperhomocysteinemia in dialysis girls/ hyperhomocysteinemia in normal adult women was 1.75/1).

In contrast to our findings, a study by Kárpáti et al [30] in 125 chronic renal failure patients on 3 mg/day folic acid supplementation showed an inverse correlation between plasma Hcy concentrations and the concentrations of folic acid and vitamin B12.

Azadibakhsh et al. [31] found a significant correlation between the mean plasma tHcy levels and mean serum folate levels; 72% of their adult HD cases had hyperhomocysteinemia. For vitamin B12, the recommended daily supplementary dose is 0.3-0.5 μg in infants and 0.5-1.5 μg in children with chronic renal failure [32,33]. The majority of our cases were supplemented with oral vitamin B12 two to four times of the recommended doses. Nakamura et al. found that functional vitamin B12 deficiency might exist even in patients with normal serum vitamin B12 concentrations and the efficacy of vitaminB12 and folate supplementation on plasma Hcy levels may depend on the MTHFR genotype [34].

Similar to our findings, Fodinger et al. [35] reported that hyperhomocysteinemia in HD patients was not related to the vitamin B12 status. They also noted that uremia, folate status, and MTHFR genotype were factors which were related to hyperhomocysteinemia. Few studies have published data on hyperhomocysteinemia in children on renal replacement therapy. Significant hyperhomocysteinemia has been reported in children and adolescents on dialysis and renal transplants with impaired renal function [36-38]. Different studies have confirmed that serum folate is the main factor affecting tHcy levels in ESRD patients [39-41].

In a case-control study, vitamin B12 and folate in HD group were not significantly different from normal controls, while the plasma vitamin B6 concentration was significantly lower than normal controls [42]. Billion et al. [2] reported folate and vitamin B12 deficiency in 10% and 6% of HD patients, respectively. In contrast to our patients, they were not supplemented with folate and vitamin B12. In addition, plasma tHcy concentrations were high in all of their patients (mean: 38.1±15 mmol/l), even in those with normal serum folate and vitamin B12 concentrations.

Similar to our patients, Vecchi et al. [43] found normal serum vitamin B12 and folate levels in the majority of their patients who were HD and peritoneal dialysis cases. The reduce risk of folate deficiency in dialysis patients has been explained by adequacy of dialysis, attention paid to dietary prescriptions, and the great availability of fresh vegetables [44,45].

Conclusion

Supplementary folate and vitamin B12 dosages of 0.5 mg/day and 3-6 μg/day in HD patients aged > 6 years result in high serum levels in the majority of patients. Although vitamin B12 and folate deficiency are suggested as etiologic factors in the pathogenesis of hyperhomocysteinemia, it seems that normal and even high serum levels of these vitamins cannot prevent the occurrence of hyperhomocysteinemia in HD patients. As folate concentrations in red blood cells and serum holo transcobalamin II are better static indices for folate and vitamin B12 deficiency respectively and measuring serum levels of MMA + plasma Hcy is a better dynamic indicator for vitamin B12 and folate status, further studies for evaluating the correlation of the red blood cell concentration of folate, serum holo transcobalamin II and serum MMA levels with plasma Hcy levels would be attractive. Comparison of these indices in patients in pre-dialysis stages (chronic kidney diseases stages I-IV) with their levels in dialysis subjects before and after supplementation with low dose folate (≤0.5 mg/day) and vitamin B12 (3-6 μg/day) can provide better information about these indices and the effect of supplementary doses on the metabolism of Hcy .

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Conflict of Interest

None declared

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