Effects Of High Dose Folate And Vitamin B12 On Hyperhomocysteinemia In Maintenance Hemodialysis Patients

Abstract

Background: Hyperhomocysteinemia is a well-recognized risk factor for accelerated atherosclerosis in hemodialysis patients. Folic acid and vitamin B12, alone or in combination have been used to reduce homocysteine (Hcy) levels in hemodialysis patients.**Objectives:** To evaluate the effect of high dose folate and vitamin B12, alone or in combination, on hyperhomocysteinemia in maintenance hemodialysis patients.**Methods:** This is a prospective interventional study and conducted at the Department of Nephrology, Dhaka Medical College Hospital, Dhaka, Bangladesh. The study included 88 patients of maintenance hemodialysis with hyperhomocysteinemia. They were randomly distributed into four equal groups by lottery method, 22 patients who received only folic acid (FA), 15mg/day for a period of 8 weeks was considered as group I, 22 patients who received only Inj. vitamin B12, 1000 µg/4 weeks for a period of 8 weeks was considered as group II, 22 patients who received combination of folic acid (FA), 15mg/day and Inj. Vitamin B12, 1000µg/4 weeks for a period of 8 weeks was considered as group III and rest22 patients who did not receive any interventionwas considered as group IV (Control). In pre- and post-intervention phases total plasma homocysteine concentration, serum folate and vitamin B12 levels were measured. Statistical analysis of the results were obtained by using window based computer software devised with Statistical Packages for Social Sciences (SPSS-22).**Results:** The mean plasma total homocysteine (tHcy) at baseline was 32.52±8.68 (µmol/L)in group I, 31.45±9.94 (µmol/L)in group II, 33.29±11.08 (µmol/L)in group III, 31.61±6.87 (µmol/L) in group IV. The mean plasma tHcy at the end of 2nd month (post intervention) reduced to 26.45±6.23 (µmol/L)in group I, 27.38±7.63 (µmol/L)in group II and 21.72±6.91 (µmol/L) in group III and 29.98±7.05 (µmol/L) in group IV. The reduced plasma tHcy at the end of 2nd month was statistically significant (p=0.001) among four groups. The mean reduction (%) of tHcy was 18.7 in group I, 12.95 in group II, 34.76 in group III and 5.16 in group IV. The mean serum folic acid at the end of 2nd month was 22.94±6.62 (ng/ml) in group I, 6.68±1.9 (ng/ml) in group II, 25.03±5.18 (ng/ml) in group III and 7.35±1.87 (ng/ml) in group IV. The mean serum vitamin B12 at the end of 2nd month was 388.14±191.55 (pg/ml) in group I, 1120.8±204.4 (pg/ml) in group II, 1066.8±228.58 (pg/ml) in group III and 385±99.05(pg/ml) in group IV. The difference of serum folic acid and serum vitamin B12 at the end of 2nd

month were statistically significant (p<0.05) among four groups. **Conclusion:** High dose folate and vitamin B12, alone or in combination were effective in reducing homocysteine level in maintenance hemodialysis patients.

Keywords: Hyperhomocysteinemia, Folate, Vitamin B12, Hemodialysis.

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Introduction

End-stage renal disease (ESRD) patients are at risk of developing coronary artery disease (CAD), peripheral vascular disease and stroke. The cardiovascular diseases remain the leading cause of mortality and morbidity in maintenance hemodialysis patients than that of the general population (10–20 fold). [1]Twenty five to sixty percent of end stage renal disease (ESRD) patients die from Atherosclerotic cardiovascular diseases (ASCVD). This high prevalence of cardiovascular mortality can't be fully explained by the classical risk factors only such as smoking, hypertension, hypercholesterolemia and diabetes mellitus. Hyper-homocysteinemia is now recognized as an independent risk factor for ASCVD in maintenance dialysis patients. This is significant because hyper-homocysteinemia is one of the most prevalent risk factors for ASCVD, which occurs in 83 to 91% of ESRD patients .[2]Hyperhomocysteinemia induces oxidative stress, inhibits antioxidant enzymes, accelerates atherosclerosis and thrombogenesis by stimulating impairment of coronary microvascular dilator function, smooth muscle proliferation, platelet activation, endothelial dysfunction and collagen synthesis .[3,4,5]Several studies have shown that hyperhomocysteinemia is an independent risk factor for atherothrombotic diseases and its role as a predictor of cardiovascular diseases especially in ESRD patients, is equivalent to the traditional risk factors. However, lowering high tHcy (Plasma Total Homocysteine) concentrations in dialysis patients is required as its higher levels may partially responsible for thrombotic disease in dialysis patients and homocysteine lowering therapy has proved to be effective in prevention of cardiovascular morbidity and mortality.[6,7,8]The patho-physiological mechanisms for hyper-homocysteinemia in renal failure are not fully known, although several explanations have been suggested but these are important to consider since these form the basis for therapy of hyper-homocysteinemia. Homocysteine is a sulphur containing essential amino acid formed during methionine metabolism. The impairment of remethylation pathway is mostly responsible for elevated fasting homocysteine level and correlates best with atherosclerotic risk. Several mechanisms have been described to explain the impairment of

the remethylation pathway in renal failure, including a subclinical deficiency of two cofactors such as folic acid and vitamin B12 which are needed in the remethylation pathway. Another possible explanation for hyper-homocysteinemia in ESRD patients is low glomerular filtration rate as the kidney has important role in the normal metabolism of homocysteine. Two recent studies have shown a marked increase in the half-life of homocysteine in ESRD patients caused by a significant reduction in the clearance of homocysteine by the kidney. This decreased homocysteine metabolism by the diseased kidneys in ESRD patients, may be potentiated by the very low levels of serine (an amino acid produced in the kidney), since serine is needed as a methyl donor in the remethylation pathway.[2,7,9,10,11]These pathways are disrupted by deficiencies of three micronutrients, two of which are cofactors (vitamins B-6 and B-12) and one of which is a substrate (folate).[12,13,14] ESRD patients on hemodialysis are at risk of developing hydrosoluble vitamin deficiency due to their poor intake, decrease absorption, altered metabolism, and/or dialysis-induced losses. High flux hemodialysis and erythropoietin therapy may also precipitate folate and vitamin B12 deficiencies. Moreover, chronic kidney disease patients with hyperhomocysteinemia have a relative resistance to the lowering effects of low dose folate supplementation. Among the moderately hyperhomocysteinemic individuals with normal renal function, 1 to 5 mg/day of folic acid may normalize plasma homocysteine level but similar effect has not been observed in ESRD patients. So, the optimal dose and the ultimate benefits remain to be clearly demonstrated in chronic dialysis patients.[15,16,17,18]Tetrahydrofolate (active form of folic acid) and vitamin B12 are involved in both the pathways of homocysteine metabolism and act as cofactors. Based on these metabolic pathways, different studies have been done to normalize plasma homocysteine level in hemodialysis patients. Most dialysis patients receive oral folic acid 5 mg/day when they are initiated on HD treatment; however, this strategy was not adequate to control hyperhomocysteinemia in them.[1,6,19]The aim of this study is to evaluate the effects of high doses of folate and vitamin B12 supplementation, alone or in combination in reducing plasma total homocysteine concentrations in maintenance hemodialysis patients.

Materials And Methods

This prospective interventional study was conducted in Hemodialysis unit of Department of Nephrology, Dhaka Medical College Hospital, Dhaka Bangladesh from January 2018-December 2018. Patients of maintenance hemodialysis with hyperhomocysteinemia in the Hemodialysis unit of Department of Nephrology, Dhaka Medical College Hospital, Dhaka

were included in this study. Total 88 patients included in our study. All the patients were recruited as per inclusion and exclusion criteria.This study investigated the effect of high dose folate and vitamin B12 on hyperhomocysteinemia in maintenance hemodialysis patients.This randomized trial was conducted on 88 (Eighty eight) maintenance hemodialysis patients with hyperhomocysteinemia. They were randomly distributed by lottery method into four equal groups: Group I, II & III were considered as intervention groups and group IV as control group.

Inclusion Criteria:

- Patients ≥18 years old who had received chronic maintenance hemodialysis for ≥ 3 months.
- Patients having serum homocysteine level > 15 μmol/L
- Patients having serum vitamin B12 levels 200-900 pg/mL

Exclusion Criteria:

- Receiving folic acid supplementation ≥ 5mg/day
- Concomitant infection or active inflammatory disorder
- Malnourished patient (serum albumin less than 2mg/dl)
- Currently taking antifolate or antiepileptic drugs.
- History of hypersensitivity to folic acid and Vitamin B12.

Study Procedure

Group I- 22 (twenty two) patients who received only oral folic acid (FA), 15mg/day for a period of 8 weeks.

Group II - 22 (twenty two) patients who received only intravenous injection of vitamin B12 (Hydroxocobalamin), 1000 μg/4weeks (postdialysis) for a period of 8 weeks.

Group III - 22 (twenty two) patients who received combination of oral folic acid (FA), 15mg/day and intravenous injection of Vitamin B12 (Hydroxocobalamin), 1000μg/4 weeks (postdialysis) for a period of 8 weeks.

Group IV (Control)- 22 (twenty two) patients who did not receive any intervention.

All the patients were receiving their routine medications including multivitamin supplements containing 0.4 mg folate, 6 mcg vitamin B12 and 2 mg vitamin B6. The dosage of anti-hypertensive, anti-diabetic agents, lipid lowering agents and antiplatelet drugs were continued and adjusted according to the individual patient's clinical condition.

Data processing and analysis: Statistical analysis of the study was done by computer software devise as the Statistical Package for Social Science (SPSS-22.0). Categorical

data was presented as frequency & percentage and continuous data as mean± standard deviation. Confidence interval was considered at 95% level. *p* value of <0.05 was considered statistically significant at 95% confidence interval.

Operational definitions:

Hyper-Homocysteinemia

- The American Heart association released an advisory statement classifying total plasma homocysteine concentration as follows:
- Normal level: 5-15 μmol/L
- Moderate hyperhomocysteinemia: 16–30 μmol/L
- Intermediate hyperhomocysteinemia: 31–100 μmol/L
- Severe hyperhomocysteinemia: > 100 μmol/L
- Therefore, hyperhomocysteinemia is defined as total plasma homocysteine level above 15 μmol/L.

Serum Folate level

- The normal level of serum folate ranges from 2-20 ng/ml.

Serum vitamin B-12 Level

- The normal level of serum vitamin B-12 ranges from 200-900 pg/ml.

Maintenance hemodialysis:

- ESRD patient on regular hemodialysis for ≥8 hours /week for at least 3 months.

Results

This study was conducted in the Department of Nephrology, Dhaka Medical College Hospital, Dhaka. Total 88 patients were taken for this study. They were divided into four equal groups randomly, consisted of 22 patients in each group: Group I, II& III were considered as intervention groups and group IV as control group. Group I - patients received only oral folic acid, 15mg/day; GroupII -patients received only intravenous injection of vitamin B12, 1000 μg/4weeks; Group III- patients received combination of oral folic acid, 15mg/day and intravenous injection of Vitamin B12, 1000μg/4 weeks; Group IV (Control)- patients did not receive any intervention.

Table 1: Distribution of the study patients according to demographic data (n=88)

	Group I N=22	Group II N=22	Group III N=22	Group IV N=22	p value
Age (years) Mean±SD	44.59 ±12.42	46.23 ±13.47	50.73 ±10.85	46.64 ±15.58	[a]0.465[ns]

Range(min-max)	22 -70	18 -71	30 -70	26 -78	
BMI (kg/m2) Mean±SD	23.75±2.96	24.05±4.25	23.99±2.26	25.06±1.53	[a]0.464[ns]
Range (min-max)	17.5-30.6	17-38.6	18.1-28.1	21.8-28.4	
Duration of HD (Months) Mean±SD	37±22	33±19	28±7	29±6	[b]0.195[ns]
Range(min-max)	3-84	3-60	3-48	3-50	
Blood pressure					
SBP (mmHg) Mean±SD	143.6±18.1	143.18±17.01	139.5±17.9	148.6±15.5	[b]0.337[ns]
Range (min-max)	120-180	120-180	110-170	120-180	
DBP (mmHg) Mean±SD	82.5±8.1	83.86±9.25	87.1±9.2	89.45±9.5	[b]0.053[ns]
Range (min-max)	70-100	70-100	80-110	80-110	

Group I= Received only oral folic acid, 15mg/day, Group II=Received only inj. of vitamin B12, 1000 µg/4weeks, Group III= Received combination of oral folic acid, 15mg/day and inj. of vitamin B12, 1000µg/4 weeks and Group IV= Control.

ns= not significant

[a]*p* value reached from ANOVA test

[b]*p* value reached from Chi-square test

Table 1 showsthe mean age was 44.59±12.42 years with ranged from 22 to 70 years in group I, 46.23±13.47 years with ranged from 18 to 71 years in group II, 50.73±10.85 years with ranged from 30 to 70 years in group III and 46.64±15.58 years with ranged from 26-78 in group IV**.** No significant difference of BMI among four groups. Male were more than female but difference was not statistically significant ($p>0.05$).

Table 2: Comparison of the study patients according to plasma total homocysteine (n=88)

Plasma tHcy(µmol/L)	**Group I (n=22)**		**Group II (n=22)**		**Group III (n=22)**		**Group IV (n=22)**		***p* value**
	Mean	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**	
Baseline	32.52	±8.68	31.45	±9.94	33.29	±11.08	31.61	±6.87	0.668[ns]
Range(min-max)	18.1	-52.1	18.1	-52.6	17.6	-54.5	20.9	-46.1	
At the end of 2nd month	26.45	±6.23	27.38	±7.63	21.72	±6.91	29.98	±7.05	0.001[s]
Range(min-max)	16.4	-38.1	17.1	-45.2	13.2	-36.2	20.6	-44.1	
Reduction (%)	18.7		12.95		34.76		5.16		

Table 2 shows the mean plasma total homocysteine (tHcy) at baseline was 32.52±8.68 (µmol/L) in group I, 31.45±9.94(µmol/L)in group II, 33.29±11.08 (µmol/L)in group III,

31.61±6.87 (µmol/L) in group IV and at the end of 2nd month was 26.45±6.23 (µmol/L) in group I, 27.38±7.63 (µmol/L)in group II, 21.72±6.91 (µmol/L) in group III and 29.98±7.05 (µmol/L) in group IV. The mean change reduction (%) was 18.7 in group I, 12.95 in group II, 34.76 in group III and 5.16 in group IV. The difference of plasma tHcy at the end of 2nd month was statistically significant (p<0.05) among four groups.

Table 3: Comparison of the study patients according to serum folic acid (n=88)

Serum folic acid (ng/ml)	**Group I (n=22)**		**Group II (n=22)**		**Group III (n=22)**		**Group IV (n=22)**		***p* value**
	Mean	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**	
Baseline	8.55	±2.56	8.75	±2.4	7.14	±3.0	8.17	±2.10	0.161[ns]
Range(min-max)	3.7	-13.3	4.9	-14.1	2.1	-12.1	3.4	-11.2	
At the end of 2nd month	22.94	±6.62	6.68	±1.9	25.03	±5.18	7.35	±1.87	0.001[s]
Range(min-max)	8.9	-41.2	4.1	-11.3	12.3	-33.6	3.1	-9.6	

Table 3 shows the mean serum folic acid at baseline was 8.55±2.56 (ng/ml) in group I, 8.75±2.4 (ng/ml) in group II, 7.14±3.0 (ng/ml) in group III and 8.17±2.10 (ng/ml) in group IV, and at the end of 2nd month was 22.94±6.62 (ng/ml) in group I, 6.68±1.9 (ng/ml) in group II, 25.03±5.18 (ng/ml) in group III and 7.35±1.87 (ng/ml) in group IV. The difference of serum folic acid at the end of 2nd month was statistically significant (p<0.05) among four groups.

Table 4: Comparison of the study patients according to serum vitamin B12 (n=88)

Serum vitamin B12 (pg/ml)	**Group I (n=22)**		**Group II (n=22)**		**Group III (n=22)**		**Group IV (n=22)**		***p* value**
	Mean	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**	
Baseline	511.8	±257.6	403.5	±152.3	384.1	±140.8	424.2	±105.5	0.081[ns]
Range(min-max)	34	-927	209	-812	207	-634	271	-971	
At the end of 2nd month	388.1	±191.5	1120.8	±204.4	1066.8	±228.5	385	±99.05	0.001[s]
Range (min-max)	148	-746	809	-1607	688	-1503	251	-604	

Table 4 shows the mean serum vitamin B12 at baseline was 511.8±257.6 (pg/ml) in group I, 403.5±152.3 (pg/ml) in group II, 384.1±140.8 (pg/ml) in group III, 424.2±105.5 (pg/ml) in group IV and at the end of 2nd month was 388.1±191.5 (pg/ml) in group I, 1120.8±204.4 (pg/ml) in group II, 1066.8±228.5 (pg/ml) in group III and 385±99.05 (pg/ml) in group IV. The difference of serum vitamin B12 at the end of 2nd month was statistically significant (p<0.05) among four groups.

Discussion

This prospective interventional study was carried out in the Department of Nephrology, Dhaka Medical College Hospital, Dhaka, from January 2018 to December 2018 with an aim to observe the effects of high dose folate and vitamin B12 on hyperhomocysteinemia in maintenance hemodialysis patients.Total 88 patients of maintenance hemodialysis were enrolled in this study. They were randomly distributed by lottery method into four equal groups, 22 patients who received only folic acid (FA), 15mg/day for a period of 8 weeks was considered as group I, 22 patients who received only Inj. vitamin B12, 1000 µg/4 weeks for a period of 8 weeks was considered as group II and 22 patients who received combination of folic acid (FA), 15mg/day and Inj. vitamin B12, 1000µg/4 weeks for a period of 8 weeks was considered as group III and rest 22 patients who did not receive any intervention was considered as group IV(Control). Baseline and post intervention investigations were done in all four groups to determine the outcome of intervention.The mean age was 44.59±12.42 years with ranged from 22 to 70 years in group I, 46.23±13.47 years with ranged from 18 to 71 years in group II, 50.73±10.85 years with ranged from 30 to 70 years in group III and 46.64±15.58 years with ranged from 26-78 in group IV**.** The difference was statistically not significant (p>0.05) among four groups. Azadibakhsh et al.,[6] found the mean age was 48.6±17.2 years, which are comparable with the current study. On the other hand, Tayebi et al.,[20] and Ossareh et al.,[21] study showed the mean age and age range were higher obtained by their study, which may be due to geographical variations, racial, ethnic differences and genetic causes.In the current study, the mean serum folic acid at baseline was 8.55±2.56 (ng/ml) in group I, 8.75±2.4 (ng/ml) in group II, 7.14±3.0 (ng/ml) in group III and 8.17±2.10 (ng/ml) in group IV. The mean serum folic acid at the end of 2nd month was 22.94±6.62 (ng/ml) in group I, 6.68±1.9 (ng/ml) in group II, 25.03±5.18 (ng/ml) in group III and 7.35±1.87 (ng/ml) in group IV. The difference of serum folic acid at the end of 2nd month was significantly (p<0.05) higher in group III followed by group I, group II and group IV. These findings are consisted with the study of Azadibakhsh et al.,[6] as they found statistically significant (p<0.05) increase of folic acid level in the groups received higher doses such as combination of folic acid, 15mg/day & vitamin B12, 1mg/day and only folic acid, 15mg/day. But they showed no significant change of folic acid level in the groups received lower doses such as combination of folic acid, 5mg/day & vitamin B12, 1mg/day and only folic acid, 5mg/day. Similar findings were also observed by Trimarchi et al.,[22]study, where they found statistically significant (p<0.05) higher level of folic acid in

the groups received higher doses of folic acid in comparison to the rest.The mean serum vitamin B12 at baseline was 511.86±257.65 (pg/ml) in group I, 403.5±152.32 (pg/ml) in group II, 384.09±140.81 (pg/ml) in group III and424.20±105.5 (pg/ml) in group IV. The mean serum vitamin B12 at the end of 2nd month was 388.14±191.55 (pg/ml) in group I, 1120.8±204.4 (pg/ml) in group II, 1066.8±228.58 (pg/ml) in group III and 385±99.05 (pg/ml) in group IV.The difference of serum vitamin B12 at the end of 2nd month was significantly (p<0.05) higher in group III followed by group II, group I and group IV. These findings are in accordance with the study of Azadibakhsh et al.,[6] and Tayebi et al.,[20] as they found statistically significant (p<0.05) increase of vitamin B12 level in the groups received higher doses of vitamin B12, such as 1mg/day and 200µg/week, respectively.The study was focused on to evaluate the effect of high dose folate and vitamin B12 on hyperhomocysteinemia in maintenance hemodialysis patients. The mean plasma total homocysteine (tHcy) at baseline was 32.52±8.68 (µmol/L) in group I, 31.45±9.94 (µmol/L) in group II, 33.29±11.08 (µmol/L) in group III and 31.61±6.87 (µmol/L) in group IV. Difference in distribution of baseline tHcy among four groups was not significant (p>0.05). The mean plasma tHcy at the end of 2nd month was 26.45±6.23 (µmol/L) in group I, 27.38±7.63 (µmol/L) in group II, 21.72±6.91 (µmol/L) in group III and 29.98±7.05 (µmol/L) in group IV. The mean change reduction (%) was 18.7 in group I and 12.95 in group II, 34.76 in group III and 5.16 in group IV. The difference of plasma tHcy at the end of 2nd month was significantly (p<0.05) lesser in group III followed by group I, group II and group IV.The percentage of reduction was more in group III followed by group I, group II and group IV.These findings are similar with the several studies on HD patients done by Naseri et al.,[23]Bostom et al.,[12]Plassmann et al.,[24]Ossareh et al.,[21]Nakhoul et al.,[25]Manns et al.,[2] and have shown that high dose folic acid and vitamin B12, alone or in combination are effective in reducing plasma homocysteine level in maintenance hemodialysis patients. But, different results were demonstrated by the studies conducted by Billion et al.[26] and Trimarchi et al.[22] Billion et al.[26] study showed that baseline plasma homocysteine level is the major predictor of the response to therapy and patients with normal levels of tHcy may show insignificant response. Trimarchi et al. [22] study found no significant change of plasma tHcy level in the co-supplemented group in comparison to the group that received only folic acid which may be due to low dose of folic acid supplementation.

Conclusion

It can be concluded that high dose folate and vitamin B12 are effective in reducing homocysteine level in maintenance hemodialysis patients.

Limitation and Recommendation

1. It was a single centered study.
2. The duration of the study may not be long enough to evaluate the clinical benefit of the reduction in homocysteine levels.
3. Sample size does not reflect the whole country scenario.Etiology of ESRD were not similar among the study patients.

Further long-term multicentric studies can be undertaken by including large number of patients.

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