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Original Article

Effect of Vitamin B_{12} Supplementation on Serum Homocysteine in Patients Undergoing Hemodialysis: A Randomized Controlled Trial

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ABSTRACT. Clinical studies have shown that hyper-homocysteinemia is a potent independent risk factor for cardiovascular diseases, and many different methods have been investigated for lowering it in hemodialysis (HD) patients. Our study investigated the effect of Vitamin B_{12} supplementation on serum homocysteine levels in these patients. This randomized trial was conducted on 140 HD patients. They were randomly distributed by lottery method into two groups: intervention and control. In the intervention group, 100 µg/mL of Vitamin B_{12} was intravenously injected two times a week, for eight weeks. No intervention was performed in the control group. Serum levels of homocysteine, hemoglobin (Hb), and hematocrit (Hct) were measured at the beginning and again after eight weeks (2 months) of treatment. About 91% of the patients had hyperhomocysteinemia (serum homocysteine >15 µmol/L). The median baseline levels of serum homocysteine in the intervention and control groups were 31.9 and 26.9 µmol/L, respectively (P = 0.1). After eight weeks, the median homocysteine level reduced significantly in the Vitamin B_{12} group to 22.2 versus 28.4 µmol/L in control group (P = 0.006). The mean Hb and Hct also changed significantly during our study (12.3 vs. 11.4 g/dL; P = 0.003 and 37.9 vs. 35.3%; P = 0.02, respectively). Our results demonstrated the existence of a statistical negative relationship between Vitamin B_{12} and serum levels of homocysteine. Detailed investigations with larger sample sizes and longer-term use of Vitamin B_{12} are recommended.

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Introduction

Patients with end-stage renal disease (ESRD) are at risk of developing vascular disease, and the rate of cardiovascular mortality and morbidity in patients undergoing hemodialysis (HD) is significantly higher than that of the general

population (10–20 fold).[1] Numerous clinical studies in recent years have shown that hyperhomocysteinemia (abnormally high levels of serum homocysteine above 15 μmol/L),[2,3] similar to hypercholesterolemia or smoking is associated with atherosclerosis and is an independent risk factor for cardiovascular diseases.[4,5] Homocysteine is a non–protein amino acid synthesized by methionine that can be recycled into methionine by B group Vitamins.[6,7]

Many retrospective, observational studies have shown that hyper-homocysteinemia accelerates atherosclerosis and development of thrombosis by inhibiting endothelial cell growth, vascular inflammation, post-injury re-endothelialization, and high-density lipoprotein biosynthesis.[8,9] It is also a common manifestation of deterioration of the glomerular filtration rate,[10] and has been associated with occurrence of blood clots, heart attacks, and strokes, although it is unclear whether hyper-homocysteinemia is an independent risk factor for these conditions.[11] Hyper-homocysteinemia is seen in 80–100% of HD patients and HD alone does not usually normalize it.[9]

Since lowering the serum homocysteine levels in HD patients may be effective in lowering thrombotic disease, different techniques have been tried for lowering its levels in HD patients.[12] Some studies have shown that folic acid and Vitamin B_{12} together are effective in lowering the plasma concentrations of homocysteine, but it is not known whether Vitamin B_{12} alone can properly lower homocysteine in HD patients.[1] Our study was conducted to explore the effect of isolated Vitamin B_{12} supplementation on serum concentrations of homocysteine in HD patients.

Materials and Methods

Design

This study was conducted by using a randomized and parallel-group trial from October 2012 to January 2013 on patients with ESRD undergoing maintenance HD.

Patients

One hundred and forty volunteer patients were enrolled from two HD units in two hospitals in an urban area of Iran. Available sampling was used to select the patients for the study. We first measured the serum levels of Vitamin B_{12} in all patients and identified the patients who did not have Vitamin B_{12} deficiency. We then distributed the samples randomly by lottery method into two groups (simple random sampling); the intervention group (seventy patients) received intravenous Vitamin B_{12} and the control group (seventy patients) did not receive any intervention (Figure 1).

The sample group included all patients with ESRD,[13] aged equal to or older than 18 years, attending regular HD, three sessions per week, had received HD ≥3 months, had serum Vitamin B_{12} levels >200 pg/mL (lack of Vitamin B_{12} deficiency), and did not take Vitamin B_{12} for at least three months before the study. Exclusion criteria included patients who had cancer, active infection, transferred to other dialysis centers, or died.

Both groups were alike in clinical particulars including age, sex, marital and employment status, duration and session of dialysis, and smoking. The length of HD sessions in all patients was four hours, and was performed three times a week with similar KT/V. The KT/V did not change during the study. Since most HD patients in Iran adhered to a special diet (according to Islamic Iranian food culture), the type of diet was similar in both groups during the study. Because the underlying cause of ESRD may influence the Vitamin B_{12} concentration, we matched both groups in this respect.

Ten of the 140 patients were excluded from the study due to the development of cancer, shifting to other dialysis centers, developing active infections, death, or their own refusal, and only 130 patients completed the study (67 persons in the intervention and 63 persons in the control group). The sample size was determined by elements such as (a) estimation of outcomes in each group; (b) the α (type I) error level; (c) the statistical power error level; and (d) the standard deviation (SD) of the measurements for continuous outcomes. In this the study, P <0.05 was viewed as significant and

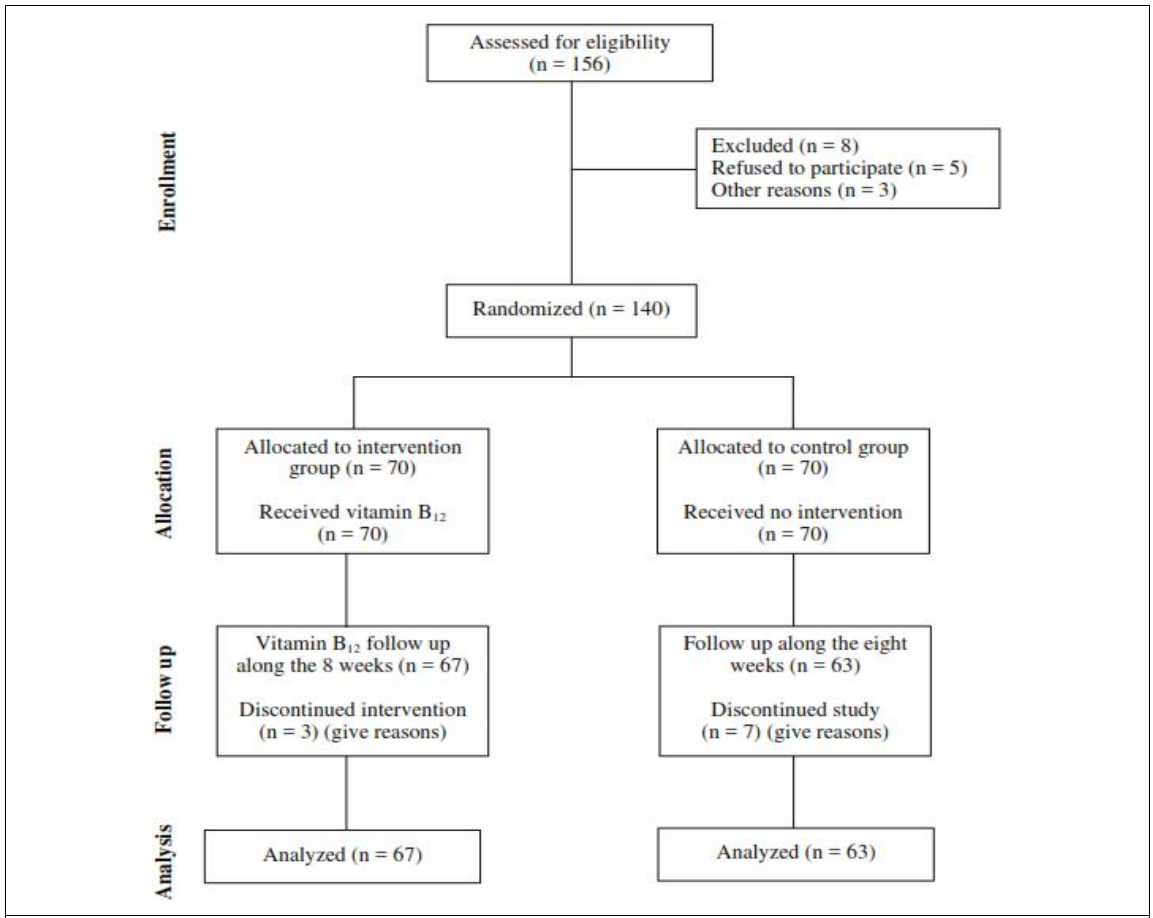

Figure 1. Consort diagram of the study groups.

statistical power was considered 80%.

Ethical approval

Verbal and written informed consent was obtained from each participant about the study purposes, confidentiality of their information, and the possibility to withdraw from the study at any stage. Ethical approval was obtained from the Institutional Ethical Committee.

Data collection

The data were gathered via demographic and measurement of the required laboratory parameters.

Instruments

A researcher-made questionnaire of demographic data was used. It consisted of age, gender, marital and education status, employment status, income, weight, smoking history, cause of nephropathy, and duration of receiving dialysis. Required laboratory parameters including serum levels of Vitamin B_{12}, hemoglobin (Hb), hematocrit (Hct), and homocysteine were measured at the beginning and at the end of the study. The blood samples were taken via the venous line at the beginning of HD sessions.

Methods

In the intervention group, two times a week for eight weeks in a row, 100 µg/mL of Vitamin B_{12} was injected immediately at the end of each HD session via the intravenous route. In the control group, no intervention was performed.

Statistical analyses

All data are shown as mean (SD). The Chi-square test, *t*-test, paired *t*-test, and ANOVA were used in this study. The data were analyzed

Table 1. Baseline qualitative characteristics of the respondents.

Characteristics	Group		*P**
	Intervention *n* (%)	Control *n* (%)	
Gender			
Male	36 (27.7)	42 (32.3)	0.09
Female	31 (23.8)	21 (16.2)	
Education			
Primary-secondary	47 (36.2)	31 (23.9)	0.06
College/university-level	20 (15.4)	32 (24.5)	
Employment			
Employed	4 (3.1)	9 (6.9)	0.99
Retired	32 (24.6)	31 (23.9)	
Housekeeper	0 (0)	16 (12.3)	
Unemployed	31 (23.8)	7 (5.4)	
Smoking			
Yes	3 (2.1)	0	0.52
No	66 (51)	61 (46.9)	

*The type of the test: Chi-square-test.

using the SPSS software version 18.0 (SPSS Inc., Chicago). P <0.05 was denoted as statistical significance.

Results

Participant characteristics

Out of the 130 respondents, 78 (60%) were male and 52 (40%) were female. The mean age of the respondents was 61.84 years (SD 12.8) (range 27–88 years). Most of the respondents (71.5%) were in the age-group of >55 years. Sixty-three (48.5%) participants were retired, 16 (12.3%) described themselves as homemaker/husband, 13 (10%) were employed, and 38 (29.2%) were unemployed. The mean age for retired, unemployed, and employed individuals was 63.6, 63, and 52.6 years, respectively. The mean age of males (62.1 years) was similar to females (61.5 years). The mean duration on HD was 40.6 months (SD 49.1; range 3–276 months). The main causes of renal disease were hypertension (HTN) and diabetes mellitus (DM) (34.9% HTN, 5% DM, 33.3% HTN and DM simultaneously). The educational level in 60% of the participants was primary-secondary. The mean serum Vitamin B_{12} level was 901.4 (SD, 602.1) pg/mL. Additional demographic characteristics of the patients are listed in Tables 1 and 2.

Incidence of hyper-homocysteinemia

The mean homocysteine level in the study group was 29.5 ± 18.3 µmol/L. Ninety-one percent of patients had hyper-homocysteinemia (serum homocysteine >15 µmol/L) and more than 30% of them had intermediate to severe hyper-homocysteinemia. Sixty-nine percent of

Table 2. Baseline quantitative characteristics of the respondents.

Characteristics	Group (mean ± SD)		*P**
	Intervention	Control	
Age (years)	63.15 ± 11.8	60.44 ± 13.6	0.23
Dialysis duration (months)	42.6 ± 57.1	38.4 ± 39.3	0.63
Body weight (kg)	68.43 ± 11.9	69.25 ± 12.7	0.8
Years married (years)	31.96 ± 11.9	32.81 ± 11.1	0.78
Children (numbers)	3 ± 1.38	3.4 ± 0.47	0.28
Serum parameters			
Red blood cells (mil/mm^3)	3.82 ± 0.4	3.67 ± 0.8	0.4

*The type of the test: Independent *t*-test. SD: Standard deviation.

men and 49% of women declared the degree of hyper-homocysteinemia. The median baseline of serum homocysteine in the intervention and control groups was 31.9 and 26.9 µmol/L, respectively (P = 0.1). After two months, the level reduced significantly in the Vitamin B_{12} group to 22.2 versus 28.4 µmol/L in the control group (P = 0.006).

Other variables

The mean of serum Hb and Hct, was respectively, 11.3 g/dL (SD 1.5) and 34.8% (SD 4.7). The mean Hb and Hct levels showed a significant change during the study period; 12.3 versus 11.4 g/dL; P = 0.003 in the intervention group and 37.9 versus 35.3% in the control group; (P = 0.02, respectively).

Relationships between variables

There was a significant and direct correlation between serum B_{12} and Hb levels (P = 0.02), which indicated that the increase of serum B_{12} led to the increase of Hb. A statistically significant relationship was found between serum Hb and Hct after intervention in both groups (P = 0.01). An independent *t*-test showed the existence of a positive statistical relationship between age and serum homocysteine >15 µmol/L (P = 0.001). There was a significant correlation between employment status and serum homocysteine (P = 0.002) as all of the unemployed patients had hyper-homocysteinemia. An independent *t*-test showed that there was no meaningful relationship between dialysis history and serum homocysteine (P = 0.2). A one-way ANOVA established that there was no statistical correlation between serum homocysteine and marital and educational status, cause of nephropathy, and income level (P >0.05). In addition, no significant relationship was found between serum Hb and homocysteine (P >0.05).

Interaction of Vitamin B_{12} with serum homocysteine

As seen in Table 3, the intervention group which received supplemental Vitamin B_{12} attained a considerable amelioration in serum homocysteine levels. An independent t-test between groups after intervention indicated that serum homocysteine decreased significantly in the intervention group (P = 0.006): baseline 31.9 (19.6) versus final 22.2 (6.7); the effect size of serum homocysteine was 0.5 and confidence interval was 10.7–1.95. This mean increased in the control group: baseline 26.9 (14) versus final 28.4 (16.6).

Discussion

We hypothesized that supplemental Vitamin B_{12} would have an effect on serum homocysteine, and designed this study to test that hypothesis. In addition, comparison between the group of patients who received supplemental Vitamin B_{12} (Group I) and the group that did not (Group II) showed the existence of a significant negative relationship between Vitamin B_{12} and serum levels of homocysteine.

Although many studies have evaluated the effect of folic acid and Vitamin B_{12} on serum homocysteine levels in the general population, these have been little explored in patients undergoing HD;[14] also, the effect of intrave-

Table 3. Alterations in the studied variables before and after the study period.

Serum variables	Time*	Group (mean ± SD)		*P**
		Intervention	Control	
Homocysteine, (µmol/L)	1	32 ± 21.5	26.9 ± 14	0.1
	2	22.2 ± 6.8	28.4 ± 16.6	0.006
Hemoglobin (g/dL)	1	11.4 ± 1.06	11.1 ± 1.9	0.2
	2	12.28 ± 1.3	11.4 ± 1.8	0.003
Hematocrit (%)	1	35.2 ± 3.2	34.5 ± 5.9	0.4
	2	37.9 ± 4.3	35.3 ± 6	0.005
Platelets ($\times 10^3$/mcL)	1	213.1 ± 65.02	198.6 ± 71.5	0.06
	2	187.4 ± 66.8	194.1 ± 64.8	0.6

*Time 1 = pretest, Time 2 = posttest, **The type of the test: Independent *t*-test. SD: Standard deviation.

nous Vitamin B_{12} alone has not been studied. Dierkes et al reported that Vitamin B_{12} supplementation in combination with folic acid decreased homocysteine concentrations in patients with ESRD, but normalization was not achieved perfectly.[15] Manns et al reported lower Vitamin B_{12} levels in HD patients and significant reduction of homocysteine levels with folic acid administration.[16] Acetylcysteine was administered intravenously in the study by Erkan et al for the treatment of hyper-homocysteinemia in HD patients and its result was reported to be significant.[17] The house study demonstrated that administration of dimercaptosuccinic acid orally did not reduce plasma homocysteine to normal levels.[18] Our study showed that intravenous administration of Vitamin B_{12} alone lowered serum concentration of homocysteine in HD patients.

According to the literature, hyper-homocysteinemia is observed in a large part of HD patients (80–100%).[19] Our study also confirmed these results. HD patients are at risk for anemia and low levels of serum water soluble vitamins.[20] Renal anemia is associated with adverse outcomes in patients on HD and should be treated with supplementary iron and erythropoiesis-stimulating agents (e.g., rHuEpo).[21] Vitamin B_{12} deficiency may also have injurious effects on HD patients and increase erythropoietin resistance.[22] Our study demonstrated that Vitamin B_{12} administration increased the means of Hb and Hct and improved anemia. The purported increase in Hb and Hct levels in the intervention group even without any Vitamin B_{12} deficiency indicates the existence of a relationship between serum B_{12} and Hb ($P = 0.02$).

According to the literature, the incidence of chronic kidney disease was higher in men and people older than 45 years. In our study, the mean age of patients was 57.7 years and 59.6% of them were older than 55 years.

Limitations of the study

Evidence to date suggests that there is a strong inverse correlation between serum levels of folate and plasma homocysteine levels. Not measuring plasma folate levels before and after the study and not specifying the patients who had folate deficiency before the study were the limitations in our study, which limit the ability to generalize the findings. Removing this limitation was not a feasible option due to the financial costs and the limitations of the laboratories.

Another limitation of this study was the duration of the study. The duration of the study may not be long enough to evaluate the clinical benefit of the reduction in homocysteine levels. Performing studies with larger sample sizes and long-term usage of Vitamin B_{12} are recommended.

Conclusion

Hyper-homocysteinemia is a common disorder in patients on HD that is still significantly under-studied. Our findings indicate that Vitamin B_{12} supplementation may be a simple and useful intervention in modifying hyper-homocysteinemia. The results from the study also established a path for further investigation with a larger sample size and long-term usage of Vitamin B_{12}.

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