The Relationship between Folic Acid and Vitamin B12 Serum Levels with High Sensitivity C-reactive Protein and Homocysteine in Chronic Hemodialysis Patients: A Cross-sectional Study

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ABSTRAK

Latar belakang: *Pemberian suplementasi asam folat (AF) dan vitamin B12 pada pasien PGK, telah secara rutin diresepkan untuk menurunkan kadar homosistein dan peradangan pada pasien PGK. Akan tetapi, belum banyak penelitian mengenai hubungan kadar asam folat dan vitamin B12 pada pasien yang menjalani hemodialisis dua kali seminggu. Penelitian ini bertujuan untuk menilai kadar folat serum dan B12 pada pasien hemodialisis kronis dan hubungannya dengan kadar homosistein dan hsCRP dalam darah.* ***Metode:*** *Studi ini merupakan studi potong lintang yang mengikutsertakan suluruh pasien hemodialisis rutin dua kali seminggu di RS Cipto Mangunkusomo, Jakarta,Indonesia. Sampel untuk pengukuran kadar asam folat, vitamin B12, homosistein, dan hsCRP diambil dari sampel darah predialisis. Subjek yang memiliki kondisi medis yang mempengaruhi hasil pemeriksaan dikeluarkan dari penelitian ini. Korelasi antar variabel dianalisis menggunakan uji korelasi Spearman.* ***Hasil:*** *Delapan puluh subjek diikutsertakan dalam penelitian ini. Pada subjek yang tidak mendapatkan suplementasi asam folat [26 (32.5%)] dan suplementasi vitamin B12 [16 (20.0%)], hanya 3,85% subjek memiliki kadar asam folat yang rendah dan tidak ada subjek yang memiliki kadar vitamin B12 yang rendah. Didapatkan adanya korelasi negatif sedang antara asam folat serum dan kadar homosistein ($p \leq 0{,}001$; $r = -0{,}42$) dan korelasi lemah antara vitamin B12 serum dan kadar homosistein ($p = 0{,}009$; $r = -0{,}29$). Pada subjek dengan risiko kardiovaskular tinggi (CRP> 3, n = 49), terdapat korelasi negatif sedang antara asam folat serum dan kadar homosistein ($p \leq 0.001$; $r = -0.561$) dan korelasi negatif yang lemah antara vitamin B12 dan homosistein ($p = 0{,}018$; $r = -0{,}338$).* ***Kesimpulan:*** *Kadar vitamin B12 dan asam folat berhubungan negative dengan kadar homosistein, terutama pada kelompok dengan risiko kardiovaskular risiko tinggi.*

Kata kunci: *asam folat, vitamin B12, kardiovaskular, mortalitas, morbiditas, hsCRP, penyakit ginjal kronis, hemodialisis.*

ABSTRACT

Background: *Folic acid (FA) and vitamin B12 treatment have been routinely prescribed to lower serum homocysteine levels and to reduce inflammation. However, no study has been conducted to determine serum folic acid (SFA) and vitamin B12 (B12) levels in patients who have twice-weekly hemodialysis. The aim of our study was to assess serum folate and B12 levels in chronic hemodialysis patients and their relationship with hsCRP and homocysteine levels.* ***Methods:*** *Our study was a cross-sectional study involving patients who had twice-weekly hemodialysis in Dr Cipto Mangunkusumo National Hospital Jakarta, Indonesia. Predialysis blood samples were taken to measure SFA, B12, homocysteine and hsCRP levels. Patients with medical conditions affecting the assays were excluded. Spearman correlation was used to compare variables.* ***Results:*** *Eighty subjects were enrolled in this study. Among those without folic acid and vitamin B-12 supplementation, only 3.85% of subjects had low folic acid levels, and none had low vitamin B12 levels. A moderate negative correlation between serum folic acid and homocysteine level ($p \leq 0.001$; $r=-0.42$) and a weak correlation between serum vitamin B12 and homocysteine level ($p=0.009$; $r=-0.29$) was found. Among the high-risk cardiovascular group (CRP>3, n=49), there is a moderate negative correlation between serum folic acid and homocysteine level ($p \leq 0.001$; $r=-0.561$) and a weak negative correlation between vitamin B12 and homocysteine level ($p=0.018$; $r=-0.338$).* ***Conclusions:*** *There is a significant negative correlation between serum vitamin B12 and folic acid with homocysteine levels, especially in high-risk cardiovascular group.*

Keywords*: Folic acid, vitamin B12, cardiovascular, mortality, morbidity, hsCRP, CKD, chronic hemodialysis.*

INTRODUCTION

Chronic kidney disease (CKD) is a resource-draining condition with poor outcomes.[1] The spectrum of CKD ranges from early to end-stage kidney disease (ESKD), which needs kidney replacement therapy (KRT). The Indonesian Renal Registry and Indonesian Ministry of Health data showed that 25 million Indonesians are affected by CKD, and hemodialysis is the most common KRT modality among this population.[2-3] Chronic kidney disease patients have a 16 to 30-fold higher mortality compared to the general population.[4,5] The most common cause of death in CKD patients is cardiovascular disease (CVD), which comprises 37% of all causes of mortality.[6]

Homocysteine is an independent risk factor for CVD. Studies have shown that homocysteine-lowering therapy could decrease cardiovascular mortality and morbidity in CKD patients.[7,8] Folic acid has an essential role in homocysteine methylation, while vitamin B12 acts as a coenzyme in the process. Theoretically, supplementation of those two could lower the homocysteine level.[9,10] C-reactive protein (CRP) is also a good predictor for mortality.[11] A small increase in serum CRP level could be detected by high-sensitivity CRP (hs-CRP) assays.[12] High serum hsCRP has been correlated with inflammation and major adverse cardiovascular events (MACE).[13]

In chronic hemodialysis patients, folic acid and vitamin B12 are two of the most routinely prescribed drug "cocktails"; however, until now, there is unclear evidence for folic acid and vitamin B12 deficiency in chronic hemodialysis patients. This practice is based on the theory that folic acid and vitamin B12 deficiency will occur in CKD. Studies in predialysis patients (i.e., CKD stage 1-4) by Hassan et al. in 2015[14] showed that folic acid deficiency increases along with kidney disease progression. In hemodialysis patients, the folic acid deficiency could be worse. Folic acid is a water-soluble nutrient with low molecular weight material. Therefore, it can be removed during the dialysis session and worsen the deficiency. Most of the published studies have demonstrated the relationship between folic acid level and all-cause mortality in hemodialysis population. However, those studies could not conclude whether supplementation of folic acid and vitamin B12 could decrease serum homocysteine level, hs-CRP, or cardiovascular events, and most of them observe the relationship in predialysis patients or thrice-weekly hemodialysis patients.[7-10 15-23] There

has not been any study involving twice-weekly dialysis patients, which are the most common type of hemodialysis patients in Indonesia.

In Indonesia, the National Health Insurance only reimburses three-weekly dialysis for a highly selected group of patients. The differences in dialysis frequency, high-flux vs. low flux dialyzers, and patient characteristics creates a demand for this kind of study. This study aims to give insight into whether folic acid and vitamin b12, a routinely prescribed supplementation, had a significant relationship with hsCRP and homocysteine, markers for a poor outcome in a twice-weekly hemodialysis population. Our study aimed to assess the level of folic acid and vitamin B12 and their relationship to hsCRP and homocysteine levels in twice-weekly hemodialysis patients.

METHODS

We performed a cross-sectional study at the Hemodialysis Unit in Cipto Mangunkusumo National Hospital, Jakarta, Indonesia, involving adult patients aged 18 years or older. The patients had been diagnosed with CKD and had undergone twice-weekly chronic hemodialysis for at least three months. We excluded patients with severe acute illness, malignancies, autoimmune disease, tuberculosis infection, exposure to immunosuppressive treatments, and gastrointestinal tract surgery history that could impair folic acid/vitamin B12 absorption. Informed consent was requested from each eligible subject for the willingness to participate in the study. Using sample size formula for correlation analysis, with alpha 0.05 and power of 80%, the minimum sample size needed for this study was 62 subjects.[24] The ethical clearance was obtained from the Research Ethics Committee Faculty of Medicine Universitas Indonesia - Cipto Mangunkusumo Hospital, Jakarta.

Demographic and clinical data

Data collection was done from August 2019 to October 2019. The patients' demographic characteristics and clinical data were collected through interviews and examination, as well as from electronic or paper-based medical records provided at the Hemodialysis Unit. Folic acid (ECLIA/ electrochemiluminescence immunoassay), vitamin B12 (CMIA/ Chemiluminescent Microparticle Immunoassay), and homocysteine (CMIA) assays were performed at the Prodia Laboratory, Jakarta, Indonesia (ISO 9001 and ISO 15189 certification). Serum hsCRP (Immunoturbidimetry) and other laboratory assays were carried out at the Department of Clinical Pathology Laboratory, Cipto Mangunkusumo National Hospital.

Statistical Analysis

Patients' baseline data, such as demographic data, clinical data, and laboratory measurements, were summarized as numerical and nominal values accordingly. Mean and standard deviations or median and interquartile ranges were used appropriately for each parameter. Spearman correlation was used to evaluate the correlation between folic acid/B12 levels and homocysteine/ hsCRP levels. A p-value of <0.05 was considered as statistically significant.

RESULTS

We screened 90 subjects. After applying inclusion and exclusion criteria, 80 subjects were included (**Figure 1**). **Table 1** summarized the baseline characteristics of the subjects. As summarized in **Table 1**, the mean age of subjects in our study was 52 (standard deviation 12.5) years. We had an almost equal number of male and female participants (38

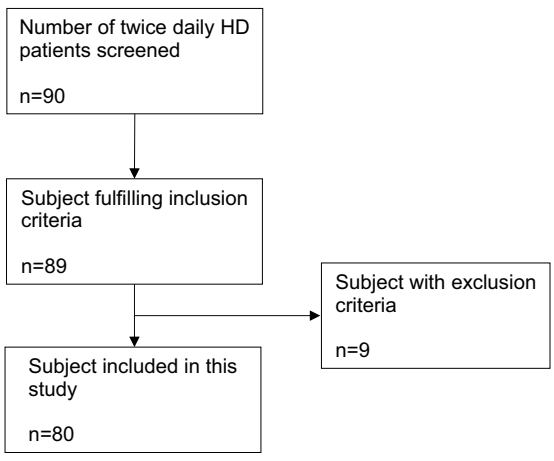

Figure 1. Flow diagram

and 42 subjects, respectively). The median duration of CKD diagnosis was 6.5 years, with a median duration of hemodialysis treatment of 5 years. The most prevalent cause of CKD was hypertension.

Results of folic acid, vitamin B12, homocysteine and hsCRp assays can be seen in **Table 2**. Normal-high B12 and folic acid levels show in 98.75% and 95% patients, respectively. Without folic acid supplementation, only 3.85% of subjects had low folic acid levels, and none of the non-vitamin B12 supplemented group had low vitamin B12 levels. About 61.3% of the subjects had high hsCRP levels. Meanwhile, high homocysteine was found in 75% of subjects and in 93.75% of subjects who have not received vitamin B12 supplementation.

Among subjects given folic acid supplementations, high hsCRP was found in 61% of subjects, while 68.5% of subjects had high homocysteine levels. Meanwhile, in subjects who were not given folic acid supplementation,

Table 1. Characteristics of subjects

Variables (N=80)	Result
Age (years), mean (SD)	52.03 (SD 12.46)
Sex (n,%)	
- Male	38 (47.5)
- Female	42 (52.5)
Duration of CKD diagnosis (years), median (IQR)	6.5 (6.75)
Duration of hemodialysis treatment (years), median (IQR)	5 (6.75)
Duration of hemodialysis (hours/session), median (IQR)	5 (0)
Qb (ml/min), median (IQR)	280 (50)
Qd (ml/min)	500
Ultrafiltration volume (ml), median (IQR)	3,700 (1,500)
Kt/V, mean (SD)	1.81 (0.36)
Weekly folate supplementation (mg/week), median (IQR)	35 (105)
- No supplementation (n,%)	26 (32.5)
- 35 mg/week (n,%)	21 (26.3)
- 70 mg/week (n,%)	8 (10.0)
- 105 mg/week (n,%)	25 (31.3)
Weekly vitamin B12 supplementation (mcg/week), median (IQR)	1,050 (4,650)
- No supplementation (n,%)	17 (21.3)
- Oral (n,%)	42 (52.5)
- Parenteral (n,%)	9 (11.3)
- Oral+parenteral (n,%)	12 (15)
BMI (kg/m^2), mean (SD)	25.06 (4.68)
Cause of CKD (n,%)	
- Hypertension	31 (38.8)
- Hypertension and diabetes	22 (27.5)
- Glomerulonephritis	9 (11.3)
- Kidney stones	5 (6.3)
- Diabetes	3 (3.8)
- Polycystic kidney disease	3 (3.8)
- Preecclampsia	2 (2.5)
- Nephrotoxic agents	2 (2.5)
- Others	2 (2.5)
- Undetermined	1 (1.3)

SD, standard deviation; IQR, interquartile range; CKD, chronic kidney disease; BMI, body mass index

Table 2. Folic acid, Vitamin B12, Homocysteine and hs CRP Measurement Results

Variables (N=80)	Total	Folic acid Supplementation N (%)		p-value	Vitamin B12 Supplementation N (%)		p-value
		No 26 (32.5%)	Yes 54 (67.5%)		No 16 (20.0%)	Yes 64 (80.0%)	
Folic acid - ng/mL (median; IQR)	18.04 (36.89)	11.65 (6.71)	27.92 (56.36)	0.001*	12.18 (6.59)	24.61 (54.9)	0.006*
- Low (N,%)	4 (5)	1 (3.85)	3 (5.56)	0.001*	0 (0)	4 (6.25)	0.003*
- Normal (N,%)	35 (43.8)	22 (84.62)	13 (24.07)		14 (87.50)	21 (32.81)	
- High (N,%)	41 (51.2)	3 (11.54)	38 (70.37)		2 (12.50)	39 (60.94)	
Vitamin B12 - pg/mL (median; IQR)	1,695 (58,286.5)	1,149.5 (3,303)	1,817.5 (145.227)	0.049*	1,017.5 (1,224)	1,817.5 (130,152.5)	0.023*
- Low (N,%)	1 (1.25)	1 (3.85)	0 (0)	0.192	0 (0)	1 (1.56)	0.246
- Normal (N,%)	22 (27.5)	9 (34.62)	13 (24.07)		7 (43.75)	15 (23.44)	
- High (N,%)	57 (71.25)	16 (61.54)	41 (75.93)		9 (56.25)	48 (75.0)	
Homocysteine - µmol/L (median; IQR)	20.85 (11.45)	22 (13.8)	20.35 (11.6)	0.122	22 (13.05)	20.7 (12.45)	0.324
- Low (N,%)	0 (0)	0 (0)	0 (0)	0.054	0 (0)	0 (0)	0.053
- Normal (N,%)	20 (25)	3 (11.54)	17 (31.48)		1 (6.25)	19 (29.69)	
- High (N,%)	60 (75)	23 (88.46)	37 (68.52)		15 (93.75)	45 (70.31)	
hsCRP - mg/L (median; IQR)	4.7 (12.63)	4.1 (7)	4.9 (13.7)	0.801	7.4 (11.85)	4.3 (12.2)	0.923
- <1,0 (N,%)	8 (10)	2 (7.69)	6 (11.11)	0.878	2 (12.50)	6 (9.38)	0.892
- 1,0-3,0 (N,%)	23 (28.7)	8 (30.77)	15 (27.78)		4 (25.0)	19 (29.69)	
- >3,0 (N,%)	49 (61.3)	16 (61.54)	33 (61.11)		10 (62.5)	39 (60.94)	

hsCRP, high-sensitivity C Reactive Protein (hsCRP); IQR, interquartile range; *) $p < 0.05$.

high hsCRP was found in 61% of subjects, and high homocysteine was found in 75% of subjects. Furthermore, in subjects given vitamin B12 supplementation, high hsCRP was found in 60.94% of subjects, while high homocysteine was found in 70.31%. In subjects who were not given vitamin B12 supplementation, high hsCRP was found in 62.5% of subjects, and high homocysteine was found in 93.8% of subjects.

We found a moderately negative correlation between serum folic acid and homocysteine level ($p \leq 0.001$; r=-0.4228) and a weak correlation between serum vitamin B12 and homocysteine level (p=0.009; r=-0.2905) (**Table 3**); however, no correlation was found between folic acid and vitamin B12 with hsCRP levels.

We performed subgroup analysis according to the subject hsCRP level to determine the high-risk group's correlation (hsCRP >3 mg/L, n=49). There is no difference found in folic acid, vitamin B12 and homocysteine levels between group (p>0.05) (**Table 4**). However, in high risk group, stronger correlations were observed both in serum folic acid (r=-0.561, p $p \leq 0.001$) and vitamin B12 (r=-0.338, p=0.018) with homocysteine level. But, no correlations were observed between vitamin B12 and folic acid with hsCRP levels (**Table 5**).

Table 3. Correlation between folic acid and vitamin B12 to hsCRP and homocysteine level

Variables	r	p-value
Folic acid and hsCRP	-0.0535	0.637
Folic acid and homocysteine	-0.4228	0.001*
Vitamin B12 and hsCRP	-0.1443	0.202
Vitamin B12 dan homocysteine	-0.2905	0.009*

hsCRP, *high-sensitivity* C Reactive Protein; *) p<0.05.

DISCUSSION

Most of our subjects had high folic acid and vitamin B12 levels. Even in those without supplementation, 96.16% of subjects had normal-high folic acid, and 100% had normal-high vitamin B12 levels. A study by Fehrman-

Table 4. Folic acid, vitamin B12 and homocysteine comparisson between cardiovascular risk group.

Variables (N=80)	Total	Cardiovascular risk		p-value
		High 49 (6.13%)	Low 31 (38.7%)	
Folic acid - ng/mL (median; IQR)	18.04 (36.89)	17 (39.01)	18.85 (28.52)	0.976
Low (N,%)	4 (5%)	3 (75.00%)	1 (25.00%)	0.785
Normal (N,%)	35 (43.8%)	22 (62.86%)	13 (37.14%)	
High (N,%)	41 (51.2%)	24 (58.54%)	17 (41.46%)	
Vitamin B12 - pg/mL (median; IQR)	1,695 (58,286.5)	1,446 (50,495)	1,918 (168,218)	0.388
Low (N,%)	1 (1.25%)	0 (0%)	1 (100.00%)	0.352
Normal (N,%)	22 (27.5%)	15 (68.18%)	7 (31.82%)	
High (N,%)	57 (71.25%)	34 (59.65%)	23 (40.35%)	
Homocysteine - µmol/L (median; IQR)	20.85 (11.45)	20.7 (10.5)	22.2 (11.3)	0.122
Low (N,%)	0 (0%)	0 (0%)	0 (0%)	0.895
Normal (N,%)	20 (25%)	12 (60.00%)	8 (40.00%)	
High (N,%)	60 (75%)	37 (61.67%)	23 (38.33%)	

IQR, interquartile range.

Table 5. Subgroup analysis in subjects according to high sensitivity C-reactive protein.

Variables	hsCRP<3.0 n=31 (38.75%)		hsCRP>3.0 n=49 (61.25%)	
	r	p-value	r	p-value
Folic acid and hsCRP	-0.141	0.439	-0,059	0,686
Folic acid and homocysteine	-0.250	0.175	-0,561	<0,001*
Vitamin B12 and hsCRP	-0.445	0.012*	0,014	0,925
Vitamin B12 dan homocysteine	-0.205	0.269	-0,338	0,018*

hsCRP, high-sensitivity C Reactive Protein; *) p<0.05.

Ekhol et al.[25] in 2008 also demonstrated a high level of vitamin B12 in the dialysis population. Many things can contribute to the elevated levels of vitamin B12 in hemodialysis patients. First, the high dose (5,000 mcg/session) of intradialytic parenteral vitamin B12 and oral vitamin B12 supplementation among the dialysis population can not be removed by dialysis. Vitamin B12 (C63H88CoN14O14P) has a molecular weight of 1,355 Daltons.[26-27] This molecule is categorized as middle molecules (500-15,000 Daltons) alongside insulin and ß-2 microglobulin and therefore, it can not be removed by dialyzer.[27]

Second, folic acid and vitamin B12 could be deposited in the body for an extended period (1-1.5 years for folic acid and 3-5 years for vitamin B12). Third, in chronic kidney disease, the production of transcobalamin II is decreased. Transcobalamin II protein functions as a vitamin B12 transporter in the small intestine.[28] Therefore, vitamin B12 uptake by tissues is reduced.[10,29]

Elevated levels of hsCRP in the majority (61.3%) of the subjects are closely related to a chronic inflammatory state. This marker was chosen because of its consistency with cardiovascular risk, comparable with systolic blood pressure and total cholesterol.[30] A study by Bazeley et al. in chronic kidney disease patients in 10 countries showed an increase in hsCRP related to increased mortality.[11] Large cardiovascular studies, such as the JUPITER study, have shown that hsCRP >2mg/L is a strong indication of therapy, and a marked decrease in cardiovascular risk was observed after therapy.[30] We also found that hyperhomocysteinemia is prevalent in most hemodialysis patients (75%). Hyperhomocysteinemia is proven to be related to arterial thrombosis and atherosclerosis. A study by Pastore et al.[31] showed that hyperhomocysteinemia occurs in more than

90% of dialysis patients.

We found that folic acid and vitamin B12 level was negatively correlated with homocysteine level, which is consistent with the results of a study conducted by Nand et al., who had such evaluation in the non-hemodialysis CKD population.[7] In the subgroup analysis, we found a moderate correlation of folic acid and homocysteine in the group of patients with hsCRP levels of > 3.0 mg/dL. We also found a mild correlation between vitamin B12 and homocysteine levels. This phenomenon could describe the protective effect that could only be seen in high-risk patients.

Our study also showed that vitamin B12 level and hsCRP was negatively correlated in low-risk patients. There is speculation that some microelements decrease during inflammation. However, a previous study reported that inflammation has no impact on the level of vitamin B12.[31.] There is also inconsistency in the results between the two groups. In the low-risk group, there was negative correlation, while the high-risk group, had positive correlation even though not statistically significant.

Selection bias in this study is unlikely to occur because the inclusion and exclusion criteria were appropriate and consistently applied in this study. Likewise, with information bias and measurement bias, those biases are unlikely to occur in this study because the data and parameters studied were measured directly and obtained from medical records.

Theoretically, we did not find potential confounders in the relationship between the variables studied. Therefore, we did not perform multivariate analysis. However, we identified a modifier effect in cardiovascular risk, which we then performed subgroup analysis. There is a possibility that our findings were found by chance. This may be due to the relatively small sample size.

Moreover, we did not measure the duration of folic acid and vitamin B12 supplementation and the variation in folic acid and vitamin B12 supplementation and intake among patients. Further research is needed to confirm these findings, and a randomized controlled trial study is recommended to see biological plausibility and time relationship.

Despite the above limitations, our study is the first study in Indonesia assessing the serum folic acid and vitamin b12 levels in the CKD population and their relationship with homocysteine and hsCRP levels. We had an 88.88% response rate which means our study can be implemented to our eligible population. However, generalization to all CKD patients needs cautions since we only included patients with stable conditions and had no comorbidity nor other conditions that can affect folic acid and B12 absorptions. In addition, we studied patients with CKD who underwent twice-weekly hemodialysis while previous studies were mostly done in thrice-weekly hemodialysis patients. We suggest conducting randomized, double-blind clinical trials to compare the benefits of each treatment.

CONCLUSION

In conclusion, there is a negative correlation between serum vitamin B12 and folic acid with homocysteine levels. Moreover, in a high cardiovascular risk subject, we found stronger correlations between folic acid and homocysteine levels and also vitamin B12 and homocysteine levels. Our findings suggest that supplementation of FA and vitamin B12 might more give benefit in high risk cardiovascular (hsCRP>3) hemodialysis patients

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper

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