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ORIGINAL ARTICLE

Folic Acid, Vitamin B6, B12 Co-supplementation Effect on Inflammatory Status of Diabetic Neuropathy Patients

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ABSTRACT

Background: Diabetic neuropathy is common in diabetes mellitus patients with the prevalence of 50%. Inflammation takes a vital role in the complex pathogenesis of diabetic neuropathy. The decreased inflammation status is becoming a potential target of therapy and prevention of diabetes complication.

Objective: to investigate the supplementation effect of vitamin B6, folic acid, and vitamin B12 in combination to inflammatory status of diabetic neuropathy patients.

Method: experimental design with randomized controlled trial. Subject of study diabetic neuropathy patients. Place of study Tugurejo Hospital, Semarang in September 2018 to March 2019, with sample size of 75 subjects (treatment and control group).

Results: Subjects of the study consists of 56 females and 19 males. The characteristic of subject in treatment and control group is not significantly different based on sex, vitamin B6, folic acid, vitamin B12 intake, and nutritional status. Compared with placebo, the effect of vitamin B6, folic acid, vitamin B12 co-supplementation is able to decrease serum Hs-CRP significantly with mean difference of -3.0 (CI 95% -4,95, -1,08; p <0,05.

Conclusion: Co-supplementation of vitamin B6, folic acid, vitamin B12 is able to significantly decrease inflamation status.

Keyword: Folic acid, vitamin B6, vitamin B12, inflammation status, diabetic neuropathy

INTRODUCTION

Diabetic neuropathy is common in diabetes mellitus (DM) patients with the prevalence of 50%.[1] Diabetic neuropathy is a range of a heterogen clinical or subclinical manifestation that affecting peripheral nerve system.[2] The general form of diabetic neuropathy mostly is symmetrical distal polyneuropathy, usually the inferior extremities are more severe than superioer extremities. The incident of the complication is increasing along with the duration of disease and the severity of hyperglycemia.[3] Diabetic neuropathy is affecting quality of life because it is the main cause of disability in consequence of foot ulceration and amputation, gait problem and trauma caused by fall.[4] Pain is experienced in around 50% neuropathy patients.[5]

The complex pathogenesis of diabetic neuropathy is characterized by metabolic and vascular factors such as hyperglycemia, mitochondrial malfunction, toxic adiposity, polyol pathway activation, oxidative stress, advanced glycation end products (AGEs) accumulation and increased inflammatiory markers.[4,6] Concentration of C-reactive protein (CRP) as one of important acute phase reactants (APR) is increased parallel with inflammation and tissue trauma.[7]

Stimulation of proinflammatory cytokines including TNF-α and IL-6 produces High-sensitivity C-reactive protein (Hs-CRP) by the liver as one of APR and found in blood.[8] Hs-CRP, sensitive marker of systemic inflammation is associated with the risk of developing diabetes type 2.[9,10] Besides, the progression of diabetic neuropathy and diabetic foot ulcer are associated with serum Hs-CRP independently. Ten percent increase in Hs-CRP from baseline value increases diabetic neuropathy as much as 1,025 (CI 95% 1,021–1,029).[11] Otherwise, the decreased events of diabetic microvascular complication that is retinopathy, nephropathy and diabetic neuropathy are significantly associated with the decreased of Hs-CRP to the moderate level (1,0-2,99 mg/L).[10]

Based on those evidences, decreased inflammatiory marked by the decreased Hs-CRP is potential target therapy in prevention and therapy of diabetic nephropathy complication.[12] Currently, tight glycemic control is still the main therapy in the prevention of diabetic nephropathy.[13–15]

Inflammation related to vascular endothelial dysfunction is strongly associated to hyperhomocysteinemia condition. A study conducted by Abdel-salam et al shows a strong correlation about Hs-CRP and homocysteine in children who underwent regular hemodialysis.[16] Another study by Li et al also stated that CRP has positive correlation with homocysteine level.[17]

Vitamin B6, vitamin B9 (folic acid), and vitamin B12 are cofactors in homocysteine metabolism. Homocysteine metabolism is consists of transulphuration and remethylation. Vitamin B6 is a cofactor in homocysteine transulphuration process, while converting homocysteine methylation into methionine involves vitamin B12 and folic acid as cofactors. These vitamin deficiencies could lead to hyperhomocysteinemia. Another studies shows that higher CRP level is associated with the low level of vitamin B6 plasma, independent from homocysteine. Vitamin B6 deficiency is related to impaired macrophage and lymphocyte-T maturation and differentiation, also inflammatory cells activation that caused the release of several enzymes and cytokines.[18] High-sensitivity C-reactive protein of acute ischemic stroke patient was reduced significantly after 14 days treatment with 5 mg folic acid and B-complex vitamin (vitamin B2 5 mg, vitamin B6

50 mg, and vitamin B12 0,4 mg). Another study shows that low dose supplementation of folic acid (400 mg/day) for 7 weeks improves vascular function by affecting endothelial nitric oxide (NO) synthase and vascular oxidative stress in patient with coronary artery disease, despite the unsignificant decrease of CRP level in few research.[7]

Studies about supplementation of vitamin B6, vitamin B9 (folic acid), and vitamin B12 to inflammatory status in patients with diabetic neuropathy are still limited and yield various results. In Indonesia, study regarding the effect of vitamin B6, vitamin B9 (folic acid), and vitamin B12 supplementation towards inflammatory status has never been done before. This reason triggers author's interest in conducting further research.

MATERIAL AND METHODS

Study design is experimental with randomized controlled trial. Sample of the study is patient with diabetic neuropathy who underwent outpatient and inpatient care in internal medicine ward Tugurejo and Ketileng Hospital in September 2018 to March 2019. The inclusion criterias are 45-65 years old diabetic neuropathy patients underwent inpatient care in internal medicine ward Tugurejo and Ketileng Hospital from January to July 2018 and adequatly treated with standard therapy. The exclusion criterias are patients having chronic kidney failure and hypothyroidism. Subjects will be considered as drop out if subject did not underwent examination and treatment procedure established in the study. There are 76 people including control and treatment group as subjects. This study has obtained ethical clearance from Diponegoro University, Faculty of Medicine, entitled No. 374/EC/FK-RSDK/V/2018 and research consent from Tugu Hospital Semarang.

Study Material and Tools: Tools used in this research are patient identity questionnaire, food frequency questionnaire, weight scale, microtoise, measuring tape, syringe and blood sample tube. Material used in this research are blood samples obtained during study, Hs-CRP reagent, combination of 1 mg folic acid, 400 ug vitamin B12, and 10 mg vitamin B6 in capsule and placebo containing 3.6 mg sodium saccharine and 45 mg of sodium cyclamate.

Study procedure: Study began by obtaining informed consent from the subjects. Subjects were interviewed about food intake by food frequency questionnaire (FFQ), smoking history, anthropometry examination and blood test for laboratory examination (serum Hs-CRP level). Subjects then treated with vitamin B6, folic acid, and vitamin B12 supplementation in combination or placebo for 4 weeks. After 4 weeks subjects are re-interviewed about food intake during the past 4 weeks, clinical evaluation of neuropathy and another blood test for laboratory examination (serum Hs-CRP level).

Data Analysis: The data was tested for the normality with Saphiro-Wilk test. The paired t-test with p <0,05 was used to analyze the difference of HsCRP level pre and post supplementation between 2 groups. The independent t-test with p <0,05 was used to analyze the difference of Hs-CRP in diabetic neuropathy patients before and after supplementation of folic acid, vitamin B6 and vitamin B12 in combination. Relationship between confounding variables (sex, smoking history, food intake and nutritional status) with dependent variables (serum Hs-CRP level) was analyzed using a correlation test with p <0,05.

RESULT

Subject: The 80 subjects in this study met the inclusion criteria from 182 screened patients (Figure 1). After randomization, 40 subjects grouped into control group and 40 subjects grouped into treatment group. After 4 weeks of treatment, 4 subjects from treatment group were excluded due to lost of follow-up and drop out.

Subject Characteristic: Subjects analyzed in this study were 56 females (74,7%) and 19 male subject (25,3%)

The following is baseline characteristic of subjects based on sex, food intake, nutritional status, homocysteine level and clinical neuropathy

Table 1 shows no significant difference of characteristic between control group and treatment group based on sex, food intake in term of vitamin B (vitamin B6, folic acid, and vitamin B12), nutritional status, and Hs-CRP level (p>0,05).

Table 1. Baseline characteristic of treatment and control group

Characteristic	Group		P
	Treatment	Control	
Sex :			
Female	25	31	
Male	11	8	0,32[a]
Food intake	125,3 ± 102,08	127,7 ± 182,38	0,42[b]
Folic acid	1,1 ± 0,57	1,2 ± 1,65	0,20[b]
Vitamin B6	17,9 ± 47,58	37,1± 88,55	0,96[b]
Vitamin B12			
Nutritional status	26,4 ± 4,19	26,1± 5,14	0,82[c]
Hs-CRP level	6,3 ± 6,09	4,9 ± 4,54	0,27[c]

Note: [a] chi-square test U,[b] Mann whitney test [c] independent t test

Table 2. Difference of the effect of supplementation on treatment group compared to control group

Variable	Treatment group				Control group				Supplementation effect	
	Pre	Post	Mean difference (CI 95%)	p	Pre	Post	Mean difference (CI 95%)	p	Mean difference of change (CI 95%)	p
Hs-CRP level	6,3 ± 6,09	4,5 ± 3,72	-1,8 (-3,59, -0,06)	0,043[a]	4,9 ± 4,54	6,2 ± 5,73	1,2 (0,22, 2,21)	0,018[a]	-3,0 (-4,95, -1,08)	0,003[b]

Note: [a] paired t test, [b] independent t test

Table 3. Correlation of other variables with Hs-CRP level

Variable	Homocysteine level	
	r	p
Sex	0,174	0,136[a]
Smoking history	-0,219	0,059[a]
Folic acid intake	0,020	0,865[a]
Vitamin B6 intake	0,043	0,713[a]
Vitamin B12 intake	-0,048	0,681[a]
Nutritional status	0,305	0,008[b]

Note: [a] Spearman's rho test, [b] Pearson correlation test

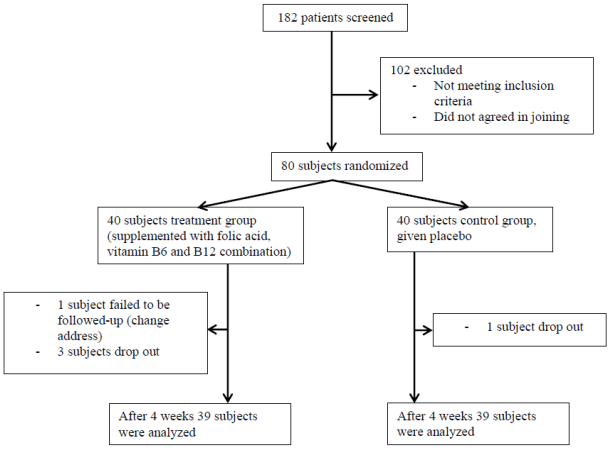

Figure 1. Subjects selection, randomisation and follow-up

DISCUSSION

Effect of vitamin B6, vitamin B9 (folic acid), and vitamin B12 co-supplementation in treatment group compared to placebo in control group could be seen in Table 2. Hs-CRP serum in treatment group after supplementation of vitamin B6, B9 (folic acid), and B12 in combination decreased significantly compared with pre-supplementation (p <0,05). The contrary results shown in control group where serum Hs-CRP increased significantly after placebo intake, compared with pre-placebo (p <0,05). Hs-CRP level in the treatment group after supplementation differ significantly compared with the control group, with mean difference -3,0 (CI 95% -4,95, -1,08) and p <0,05. This result is in accordance with study conducted by Chang et al to patients underwent hemodialysis, where serum CRP decreased significantly after treatment of 5 mg folic acid and vitamin B-complex supplementation for 3 months.[19] Recent meta-analysis also shows that supplementation of folic acid is able to decrease serum CRP significantly with mean duration more than 8 weeks of treatment. High-sensitivity C-reactive protein of acute ischemic stroke patients was reduced significantly after 14 days treatment with B-complex vitamin (vitamin B2 5 mg, vitamin B6 50 mg, and vitamin B12 0,4 mg) and 5 mg folic acid.[7] Meanwhile, this result has different result with a study conducted by Asemi et al who shows that 5 mg folic acid for 6 months does not have a significant effect to Hs-CRP level in cervical cancer.[20]

Table 3 shows that sex and smoking history are not significantly correlated with Hs-CRP level (p >0,05). This is not in accordance with the study conducted by Garcia et al in subjects with metabolic syndrome risk factors, stated that Hs-CRP level in females are higher than males. This is due to estrogen level that plays role in inflammatory process in female. Moreover, total adipose tissue amount in females is much more than males, where adipose tissue is the source of pro-inflammatory cytokines.[21] The result of this study is also not similar with Jamal et al who showed smoking individuals has Hs-CRP 55% higher than non-smoking individuals.[22] The unsignificant result found in this study might be caused by the mostly female subjects who seldom smokes. The limitation of this study is not assessing smoke exposure as passive smoker.

Table 3 also shows that folic acid, vitamin B6, vitamin B12 intake are not significantly correlated with Hs-CRP level (p >0,05). This result is not in accordance with study by Friso et al who shows association between low vitamin B6 in the plasma and higher CRP level.[18] Vitamin B6, folic acid, and vitamin B12 serum level were determined by the intake from food sources. Human body could not synthesize folate. Folate-rich food sources are yeast, liver, green vegetable, cruciferous vegetables such as broccoli. While milk, meat, fish contains folate in moderate amount, and fruits contain little amount of folate. [23] Vitamin B6 is found naturally in various food sources such as fish, meat, vegetables, fruits and also produced by gut microflora.[23] Vitamin B12 (cobalamine) is synthesized by certain microorganism and contained in several animal products such as fish, beef, poultry and dairy. The supply of vitamin B12 in human depends on food source.

Nutritional status has positive and significant correlation with Hs-CRP level (p <0,05). This result is in accordance with a study conducted by MacKenzie et al who shows that Hs-CRP increased level is associated with higher body mass index (BMI) that is higher in diabetic type 1 children. This is also in accordance with a study by Farooq et al which stated that BMI and Hs-CRP level are correlated positively.[24] Secretion of pro-inflammatory cytokines is increased in obesity individuals, while the anti-inflammatory cytokines that secreted from adipose tissue was decreased, thus resulting in elevated pro-inflammatory cytokines (interleukin-6 and TNF-alpha) and systemic inflammation. This is in accordance with diabetic neuropathy pathogenesis in obesity which the occurred inflammation could lead to central and peripheral sensitization in pain transmission system, resulting in allodynia hyperalgesia. Patients with neuropatic pain also experienced this symptoms. As long as the lesion is on the peripheral nerves, pro-inflammatory cytokines will furtherly impair axonal damage and neuronal demyelination. This is the reason why systemic inflammation which is induced by obesity will amplify neuropathic and paroxysmal pain symptoms.[21,24]

CONCLUSION

Based on this study, supplementation of folic acid, vitamin B6, vitamin B12 combination is able to significantly decrease inflammatory status in diabetic neuropathy patients. Further research is needed to review the anti-inflammatory effect of folic acid, vitamin B6 and vitamin B12 in details.

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