Effect of B vitamins supplementation on cardio-metabolic factors in patients with stable coronary artery disease: A randomized double-blind trial

doi: 10.6133/apjcn.202004/PP.0005
Published online: April 2020

Running title: Effect of B vitamines on SCAD patients

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ABSTRACT

Background and Objectives: This study aimed to evaluate whether B vitamins supplementation would improve dyslipidemia, alleviate inflammatory state of patients with stable coronary artery disease (SCAD). **Methods and Study Design:** We conducted a randomized, double-blind, 12-week, placebo-controlled trial involving adults with SCAD, and who were randomly divided into B vitamins group (folic acid and VB6) and control group (placebo tablet). Blood tests had also been performed at baseline and endpoint. **Results:** After 12 weeks of intervention, B vitamins supplementation significantly improved the concentration of serum TG, TC and HDL-C (p<0.05). Changes of serum homocysteine in B vitamins treatment were significantly different compared to placebo by the multivariate-adjusted analysis (3.02±2.35 vs 1.55±1.58 p<0.001). Meanwhile, the levels of IL-1β and IL-10, significant difference were observed between two groups after adjustment for confounding factors. **Conclusions:** Supplementation with B vitamins significantly improves lipid metabolism, alleviate inflammation and serum homocysteine concentration in patients with SCAD.

Key Words: B vitamins, SCAD, dyslipidemia, inflammatory factors

INTRODUCTION

Coronary artery heart disease (CAD) is generally regarded as heart disease caused by coronary artery occlusion myocardial ischemia, hypoxia, coronary artery, and functional changes (spasm). As one of the leading cause of death worldwide, CAD and CAD-related diseases have caused 8 million deaths worldwide in 2013, and this number would increase to 11.1 million by 2020.[1] For Chinese population, it has been estimated that there were 11 million have CAD or with CAD history.[2] The incidence of CAD is increasing by years, and the most common type is stable coronary artery disease (SCAD), seriously affecting people's quality of life in China.[3] Therefore, it is very necessary and urgent to take effective measures to prevent recurrence and aggravation of the disease and focus on the secondary prevention. Assessment of the risk of events among patients with SCAD mainly focused on clinical characteristics and biomarkers, such as lipidemia, glycemia, and inflammatory biomarkers 1.[4]

In recent years, plenty of researches had also attached importance to effects of homocysteine (Hcy) on cardiovascular and cerebrovascular disease. Substantial studies have shown that hyperhomocysteinemia caused by abnormal metabolism of Hcy is an independent risk factor of CAD.[5,6] As a generally used nutritional intervention, previous randomized

controlled trials (RCTs) have showed that B vitamins supplementation significantly reduced the levels of Hcy in patients with cardiovascular diseases.[7,8] Besides, B vitamins supplementation can also significantly improve endothelial dysfunction in patients with CAD, and were linked to protection against inflammation.[9,10] Moreover, in the Linlin Wang's study, B vitamins supplementation significantly increased the level of high-density lipoprotein cholesterol (HDL-C) in healthy subjects.[11]

However, few trials have focused on the effect of supplementation B vitamins in SCAD patients on blood lipids, especially for Chinese populations. Therefore, the present study aimed to investigate B vitamins supplement on blood lipids and inflammatory cytokines in Chinese SCAD patients with a double-blind placebo-controlled trial. We hypothesized that treatment with B Vitamins have a lowering effect on serum lipids and several inflammatory cytokines in patients with SCAD.

MATERIALS AND METHODS

Participants

In total, 122 participants aged between 45 and 80 with SCAD history were recruited from December 2014 to March 2016 from 3 centers, which were outpatient cardiology Department in Qingdao Fu Wai Cardiovascular Disease Hospital, Qingdao Community Health Service Center of Lao Shan District, and Qingdao Zhong Ren Health Management Company. The inclusion criteria were (1) diagnosed by the Chinese society of cardiology criteria for stable coronary artery disease (SCAD) (2) without taking vitamins and mineral or other supplementation three months ahead of the screening test. The exclusion criteria were (1) having acute coronary syndrome, percutaneous coronary intervention or coronary artery bypass grafting, percutaneous or surgical revascularization within the previous 3 months; (2) patients who have acute infection, or severe liver and/or kidney disease, or tumor, or peripheral vascular disease, or recently underwent a surgery; (3) patients with disabilities or showed unwillingness to comply with study treatment. All participants provided written informed consent. The research was approved by the medical ethics committee of Qingdao Disease Prevention and Control Centre, Qingdao, People's Republic of China (NO.201409). This trial was registered in the Chinese Clinical Trial Registry (ChiCTR-IOR-15006804).

Study design

The study was a randomized, double-blind, placebo-controlled trial. Subjects were randomly allocated to one of the two treatments by computer-generated random numbers, namely B vitamins group and placebo group.

Interventions

The participants in B Vitamins group were required to take two oral tablets, which included 400μg/d folic acid and 10.0 mg/d VB6 per day. The placebo group received the same regimen of placebo tablets that were identical in appearance to the B vitamins tablets. The placebo tablets contain no active ingredients (folic acid, VB6), but the other components, product specifications, packaging, taste, color and other ingredients are all in line with intervention nutrients. All participants were requested to avoid supplements, which contained folic acid and vitamin B6 during the intervention. All participants received clinical treatment, including medication, diet instruction and guidance of physical activity, and different interventions.

Measurements

The baseline characteristics of the subjects had been collected, including gender, age, body height, body weight, waist circumference, hip circumference, blood pressure (BP), heart rate (HR), and smoking habits. BP and HR were measured by electronic sphygmomanometer (OMRON, HBP9020) when the participants kept calm in a quiet environment. The waist to hip ratio was used to define body fat distribution. Patients' demographic characteristic, such as age, lifestyle factors, smoking consumption, and clinical information were obtained during a face to face interview with well-trained researchers. After 10 h fasting blood samples were collected into vacuum tubes from the antecubital fossa at baseline and endpoint. Serum was separated by centrifugation (3000 rmp for 15 min at 4 °C) and stored at -80 °C refrigerator for further analysis. Serum lipids, including triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were evaluated using enzymatic method by automatic biochemical analyzer test. Immunoturbidimetric assay was used to determine hypersensitive c-reactive protein (hs-CRP). Hcy level was detected with high performance liquid chromatography (HPLC). Enzyme-linked immune sorbent assay (ELISA) was used to detect the expression levels of interleukin (IL)-6, IL-1β, IL-4, IL-10, tumor necrosis factor-α (TNF-α), and adiponectin (APN). During the intervention, participants were interviewed on monthly basis by trained researchers in

either a hospital or a community clinic. Information on participants' lifestyle, disease progression, as well as adherence to the intervention, was collected during the interview.

Sample size

Serum levels of Hcy were regarded as the primary outcome in the present study,[12,13] as shown in a previous study. We assumed a detectable mean difference in Hcy changes of 13.0±2.1 vs. 12.9±3.3 between intervention and control groups, and considered a power of 80%, then the significant level was set at 0.05. Assuming 20% dropouts in each group, a final sample size of at least 43 persons per group was determined to enable adequate power to assess the primary outcome.

Statistical analysis

All statistical analyses were performed by SPSS 22.0, and *p*-values <0.05 (2-sided) was considered as statistical significance. Continuous data were presented as means±standard deviation (SD), while the skewed data were presented as the median (quartile range). For univariate analysis, the differences in the continuous and categorical variables were performed by using Student's t-test and chi-square test, respectively. Besides, t-test was used to estimate the intervention effects of clinical characteristics before and after intervention. To assess the supplement effects on outcomes between groups, a general linear regression model (GLM) was used to test the difference in blood lipids and inflammatory cytokines, with adjustment for age, gender, BMI, BP, WHR, smoking habits, CAD family history.

RESULTS

Baseline characteristics

In total, 99 subjects were included in this trial (62.40±8.80, 38.9% male), (Figure 1) and they were randomly assigned one of 2 groups: B vitamins group (n=46) and control group (n=53). Of these, 9 participants withdrawn from the study, including 5 persons refused to go back at the end of the trial, 3 persons were disengaged because of relocation or abroad, and 1 person died, leaving 90 participants who completed this study.

Non-significant difference was detected between two groups with respect to demographic and lifestyle characteristics, biochemical parameters, except for age (p =0.036) (Table1).

Effects of B vitamins on the serum lipids of the participants

A comparison with placebo for serum lipids changes in the group of patients receiving B vitamins supplement was presented in Table 2. After 12 weeks of intervention, B vitamins supplementation significantly reduced the concentrations of serum TG, TC and HDL-C (p<0.05). In addition, the net changes were statistically different in comparison with control group. In the multivariate regression analyses with adjustments for baseline values including gender, age, BMI, SBP, DBP, waist to hip ratio, changes in TC TG and HDL-C (p<0.05).

Effects of B vitamins on the serum inflammatory factors of the participants

The patients with B vitamins displayed a marked reduction in Hcy (p<0.05), and the multivariate-adjusted differences were also significant in B vitamins treatment compared to placebo (3.02±2.35 vs 1.55±1.58 p<0.001).

The intention-to-treat of B vitamins supplementation on the serum inflammatory factors was presented in Table 3. The serum pro-inflammatory factors were decreased in B vitamins group. Especially, the levels of IL-1β were significantly reduced in B vitamins group in comparison with placebo group in the multivariate regression analyses with adjustments for confounding factors. However, there was not significant difference with respect to APN levels in the B vitamins group after 12 weeks, compared with control group (Table 3). Regarding IL-10, significant difference was observed between B vitamins group and control group after adjustment for confounding factors (-5.13±3.80 vs -3.20±4.13，p<0.05).

DISCUSSION

In the present study, the effects of B vitamins intervention on cardio-metabolic factors were systematically investigated in patients with SCAD. The findings of this study indicated supplementation with folic acid 400μg/d and VB6 10.0 mg/d per day significantly increased HDL-C, IL-10, and reduced TG, TC, Hcy, IL-1β in SCAD patients.

Regarding risk factors of cardiovascular disease, it is generally accepted that hyperlipidemia is considered as the most important factor associated with the initiation and development of CAD.[14] Especially for SCAD patients, HDL lowering treatment could reduce MI or strokes, potentially lethal complications either acutely or subsequently.[15] However, few studies focused on supplemental B vitamins on serum lipids, which was the most concerned indicators in the treatment of SCAD patients. In this trial, serum HDL-C increased 16.7% in patients supplementation with B vitamins. Similarity, Imamura A et al. demonstrated that one

month folic acid supplementation significantly increased HDL-C levels by 6% ($p<0.05$) in healthy postmenopausal Italian women. One possible explanation was that B vitamins supplement might be related to improvement of hepatic metabolism and autoxidation.[16,17] Furthermore, we observed a significant decrease in serum level of TG and TC in B vitamins intervention group, they were respectively decreased 22.30%, 8.41% compared baseline ($p<0.05$). Based on our study, we assumed that B vitamins supplementation could attenuate lipid disorders of SCAD. Elevated serum TG and TC were independent risk factors for the morbidity and mortality of SCAD in primary prevention.[17-19] Therefore, B vitamins supplementation could effectively improve cardiovascular events and quality of life in the patients with SCAD.

Inflammation is the factor that causes the occurrence and development of SCAD. In our study, pro-inflammatory and anti-inflammatory factors were evaluated, respectively. Several studies have showed that Hcy may serve as an inflammatory marker.[20,21] The Hcy-lowing effect of B vitamins supplementation has been reported in some reviews and meta-analyses. A meta-analysis of 25 randomized controlled trials involving 2596 hyperhomocysteinemia subjects, assessed the effect of B vitamins (folic acid supplements, with or without the addition of vitamins B12 or B6) intervention on plasma Hcy concentrations, the result revealed daily 0.2-5.0 mg folic acid supplement could lower total Hcy concentrations by 13-25%.[22] Similarly, the benefit effect of folic acid and vitamin B6 was conformed in our study. In the study, the participants were all from community hospitals and discharged patients, so the serum Hcy concentration was stable at a lower baseline (12.81 μmol/L). Nonetheless, A 12-week B Vitamins intervention could effectively reduce Hcy concentrations by 23.60% in patients SCAD.

Studies have found that abnormal levels of inflammatory factors are directly related to the occurrence of CAD.[23,24] Toll-like receptors (TLRs) are important pattern recognition receptors that can specifically recognize pathogens and stimulate early immune response. After activation of TLRs, cells can secrete a variety of immune-related cytokines, among which TNF-α and IL-1β are the most important pro-inflammatory factors in the early inflammatory response and play an important role in the inflammatory response of coronary heart disease. IL-1β is associated with coronary endothelial dysfunction in patients with mTOR-inhibitor-eluting stent implantation. Moreover, IL-1β is a crucial cytokine that induced a network of signaling pathways, IL-1β plays a distinct role in neointimal formation and smooth muscle cell proliferation.[25] B vitamins supplement could effectively decrease the serum IL-1β levels in our study, one possible explanation was B vitamins could improve endothelial function.

As an inflammation cytokine, hs-CRP is related to the primary and secondary prevention of CAD. [26,27] Compared with baseline, participant in B vitamins intervention and placebo had significantly decreased serum hs-CRP, however, the changes between two groups with adjustments for baseline values had not significant difference. In contrast, some researchers observed B vitamins supplement could effectively decrease hs-CRP level in the patients with metabolic disease and cardiovascular disease.[28,29] The difference with previous studies might be due to the enrolled participants with relatively low baseline hs-CRP (the mean concentration was 1.34 mg/L) in the 2 groups.

IL-10, a protein consisting of 160 amino acid residues, is an important negative regulatory cytokine. It can inhibit the production of pro-inflammatory cytokines such as TNF-α, IL-6 and IL-1β, the activation of monocytes and T cells, and inhibit the production of macrophages and monocytes NO. Studies had demonstrated that IL-10 is an anti-inflammatory cytokine, which is associated with a humoral immune response and could limit the local inflammatory response, provide stability to the atherosclerotic lesion.[30] In this experiment, IL-10 concentration was significantly decreased in the SCAD patients group. This result means that B vitamins supplementation can improve inflammatory processes in SCAD patients.

Our trial had several strengths. First, our study demonstrated that in patients with well-controlled SCAD the intervention with B vitamins could improve Hcy concentration as well as lipid metabolism, inflammatory status. Second, the determination of B group vitamin dosing was made with reference to RDA and clinicians，and did not cause serious adverse effects. Third, this study included enough samples and implemented effective quality control to ensure compliance of participants.

There are still several limitations in our study. First, the study was performed in moderately SCAD elderly individuals, therefore our results cannot be translated directly to the general CAD population. Second, the lack of food frequency questionnaires providing data on B vitamins intake might also be considered a limitation. Third, modest sample size (less than one hundred participants) was still too small for us to examine the effect of B vitamins supplementation on SCAD.

Conclusions

This study demonstrated that supplementation with B vitamins significantly improve lipid metabolism, inflammatory status and Hcy concentration in patients with well-controlled

stable coronary artery disease. Supplemental B vitamins could be regarded as safe and effective adjuvant therapy for stable stages of SCAD patients.

CONFLICT OF INTEREST AND FUNDING DISCLOSURE

The authors declare no conflict of interest.

The study was supported by 2014 Chinese Nutrition Society (CNS) Nutrition Research Foundation-DSM Research Fund (No.2014-036).

REFERENCES

1. World Health Organization. World Health Report 2002: Reducing risks, promoting healthy life by World Health Organization. 2003;16:230. doi: 10.1080/1357628031000116808
2. GBD 2013 Mortality and Causes of Death Collaborators. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2015;385:117-71. doi: 10.1016/s0140-6736(14)61682-2.
3. Chen WW, Gao RL, Liu LS, Zhu ML, Wang W, Wang YJ et al. Summary of China cardiovascular disease report 2016. Chinese Circulation Journal. 2017;32:521-30. doi: 10.3969/j.issn.1000-3614.2017.06.001.
4. Kahan T, Forslund L, Held C, Bjorkander I, Billing E, Eriksson SV, Nsman P, Rehnqvist N, Hjemdahl P. Risk prediction in stable angina pectoris. Eur J Clin Nutr. 2013;43:141-51. doi: 10.1111/eci.12025.
5. Boushey CJ, Beresford S A, Omenn G S, Motulsky A G. A Quantitative assessment of plasma homocysteine as a risk factor for vascular disease. JAMA. 1995;274:1049-57. doi: 10.1001/jama.1995.03530130055028.
6. Guldener CV, Donker AJ, Homocysteine and atherothrombosis. N Engl J Med. 1998;338:1042-50. doi: 10.1056/NEJM199804093381507.
7. Wang Y, Jin Y, Wang Y, Li L, Liao Y, Zhang Y, Yu DJM. The effect of folic acid in patients with cardiovascular disease: A systematic review and meta-analysis. Medicine. 2019. Sep;98(37):e17095. doi 10.1097/MD.0000000000017095.
8. Ciaccio MJT, Management CR. Therapeutical approach to plasma homocysteine and cardiovascular risk reduction. Ther Clin Risk Manag. 2008;4:219-24. doi: 10.2147/tcrm.s1807.
9. Morris MS, Sakakeeny L, Jacques PF, Picciano MF, Selhub J. Vitamin B-6 intake is inversely related to, and the requirement is affected by, inflammation status. J Nutr. 2010;140:103-10. doi: 10.3945/jn.109.114397.
10. Liu Y, Tian T, Zhang H, Gao L, Zhou X. The effect of homocysteine-lowering therapy with folic acid on flow-mediated vasodilation in patients with coronary artery disease: A meta-analysis of randomized controlled trials. Atherosclerosis. 2014;235. doi: 10.1016/j.atherosclerosis.2014.03.032.

11. Wang L, Li H, Zhou Y, Jin L, Liu J. Low-dose B vitamins supplementation ameliorates cardiovascular risk: a double-blind randomized controlled trial in healthy Chinese elderly. Eur J Nutr. 2015;3:455-64. doi: 10.1007/s00394-014-0729-5.
12. Lan X, Dang SN, Zhao YL, Yan H, Yan H. Meta-analysis on effect of combined supplementation of folic acid, vitamin B12 and B6 on risk of cardio-cerebrovascular diseases in randomized control trials. Zhonghua Liu Xing Bing Xue Za Zhi. 2016;7:1028-34. doi: 10.3760/cma.j.issn.0254-6450.2016.07.024.
13. Galan P, Kesse-Guyot E, Czernichow S, Briancon S, Blacher J. Effects of B vitamins and omega 3 fatty acids on cardiovascular diseases: a randomised placebo controlled trial. BMJ. 2010;341:c6273. doi: 10.1136/bmj.c6273.
14. Benn M. Apolipoprotein B levels, APOB alleles, and risk of ischemic cardiovascular disease in the general population, Atherosclerosis. 2009;206:17-30. doi: 10.1016/j.atherosclerosis.
15. Nicolas D, Tabassome S. High-intensity lipid lowering regimens in patients with stable coronary artery disease: the intriguing question of all-cause mortality. Eur Heart J Cardiovasc Pharmacother. 2019;20. doi: 10.1093/ehjcvp/pvz049.
16. Villa P, Perri C, Suriano R, Cucinelli F, Lanzone A. L-folic acid supplementation in healthy postmenopausal women: Effect on homocysteine and glycolipid metabolism. J Clin Endocrinol Metab. 2005;90:4622-9. doi: 10.1210/jc.2004-1954.
17. Sarwar N, Danesh J, Eiriksdottir G, Sigurdsson G, Wareham N, Bingham S, Boekholdt SM, Khaw KT, Gudnason V. Triglycerides and the risk of coronary heart disease: 10,158 incident cases among 262,525 participants in 29 Western prospective studies. Circulation. 2007;115:450-8. doi: 10.1161/CIRCULATIONAHA.106.637793.
18. Yang SH, Du Y, Li XL, Zhang Y, Li S, Xu RX et al. Triglyceride to high-density lipoprotein cholesterol ratio and cardiovascular events in diabetics with coronary artery disease. 2017;354:117-24. doi: 10.1016/j.amjms.2017.03.032.
19. Emerging Risk Factors Collaboration, Di Angelantonio E, Sarwar N, Perry P, Kaptoge S, Ray KK, Thompson A et al. Major lipids, apolipoproteins, and risk of vascular disease. JAMA. 200918:1993-2000. doi: 10.1001/jama.2009.1619.
20. Misra H P. Generation of superoxide free radical during the autoxidation of thiols. J Biol Chem 1974;249:2151-5 doi: 10.0000/PMID4206550.
21. Imamura A, Murakami R, Takahashi R, Cheng XW, Numaguchi Y, Murohara T ,Okumura K. Low folate levels may be an atherogenic factor regardless of homocysteine levels in young healthy nonsmokers. Metabolism. 2010;59:728-33. doi: 10.1016/j.metabol.2009.09.017.
22. Homocysteine Lowering Trialists' Collaboration. Dose-dependent effects of folic acid on blood concentrations of homocysteine: a meta-analysis of the randomized trials. Am J Clin Nutr. 2005;82:806e12. doi: 10.1093/ajcn/82.4.806.

23. Guven M, Ismailoglu Z, Batar B, Unal S , Onaran L, Karadag B,Ongen Z. The effect of genetic polymorphisms of TLR2 and TLR4 in Turkish patients with coronary artery disease. Gene. 2015;1:99-102. doi: 10.1016/j.gene.2014.12.047.
24. Ait-Oufella H, Taleb S, Mallat Z, Tedgui A. Recent advances on the role of cytokines in atherosclerosis. Arterioscler Thromb Vasc Biol. 2011;5:969-79. doi: 10.1161/ATVBAHA.110.207415.
25. Calkin AC, Tontonoz, P. Liver X Receptor Signaling Pathways and Atherosclerosis. Arterioscler Thromb Vasc Biol. 20108:1513-8. doi: 10.1161/ATVBAHA.109.191197.
26. Ridker PDPM, Danielson E, Fonseca FA, Genest J, Glynn R. Reduction in C-reactive protein and LDL cholesterol and cardiovascular event rates after initiation of rosuvastatin: a prospective study of the JUPITER trial.Lancet. 2009;373: 1175-82. doi: 10.1016/S0140-6736(09)60447-5.
27. Liao JK. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med. 2008;359:2195-207. doi: 10.1007/s11883-009-0037-3.
28. Gariballa S, Afandi B, Abuhaltem M, Yassin J, Habib H, Ibrahim W. Oxidative damage and inflammation in obese diabetic Emirati subjects supplemented with antioxidants and B-vitamins: a randomized placebo-controlled trail. Nutr Metab (Lond). 2013;1:21. doi: 10.1186/1743-7075-10-21.
29. Ullegaddi R, Powers HJ, Gariballa SE. Antioxidant supplementation with or without B-group vitamins after acute ischemic stroke: a randomized controlled trial. J Parenter Enteral Nutr. 2006;2:108-14. doi: 10.1177/0148607106030002108.
30. Thusen V D, Journal J, Fekkes ML, De Vos P, Van Berkel TJ, Biessen EA. Attenuation of atherogenesis by systemic and local adenovirus-mediated gene transfer of interleukin-10 in LDLr -/- Mice. FASEB J. 2001;15:2730-2. doi: 10.1096/fj.01-0483fje.

Table 1. Summarize the baseline characteristics of the participants

	B vitamins group (n=42)	Control group (n=48)	*p*- values
Men, n (%)	28 (66.67)	29 (60.42)	0.539
Age (y)	60.33±9.2	64.21±8.1	0.036
BMI (kg/m^2)	70.36±10.02	69.72±11.24	0.664
Weight (kg)	26.55±3.37	26.24±3.34	0.778
WHR	0.94±0.05	0.95±0.05	0.284
SBP (mmHg)	133.69±11.05	136.88±19.56	0.354
DBP (mmHg)1	84.29±6.11	81.88±10.4	0.191
CAD family history, n (%)	10 (23.81)	13 (27.08)	0.722
Smoking, n (%)	23 (54.76)	25 (52.08)	0.799

BMI: body mass index; WHR: waist to hip ratio; SBP: systolic blood pressure; DBP: diastolic blood pressure.
Continuous values are presented as mean±SD, and categorical variables are presented as number (%). *p* values were calculated by chi-square test (for categorical variables) or independent-samples t test (for continuous variables) to test the difference between the two groups at baseline.

Table 2. Utility of treatments in the serum lipids

	B vitamins group (n=42)			*p*-values†	Control group (n=48)			*p*-values†	*p*-values‡	*p*-values§
	Time				Time					
	Baseline	Endpoint	Change		Baseline	Endpoint	Change			
TG (mmol/L)	1.84±0.73			0.001	1.96±0.69			0.082	0.436	0.378
		1.43±0.63				1.80±0.65			0.008	0.005
			0.41±0.30				0.16±0.64		0.008	0.015
TC (mmol/L)	5.47±0.98			<0.001	5.19±1.30			<0.001	0.247	0.102
		5.01±0.93				5.12±1.29			0.673	0.120
			0.46±0.23				0.07±0.05		<0.001	<0.001
HDL-C (mmol/L)	1.32±0.33			0.013	1.30±0.28			0.037	0.678	0.372
		1.54±0.60				1.43±0.42			0.096	0.047
			-0.22±0.54				-0.14±0.44		0.017	0.020
LDL-C (mmol/L)	3.16±0.94			0.07	3.34±0.75			0.828	0.333	0.197
		2.88±0.61				3.32±0.71			0.001	<0.001
			0.29±0.97				0.01±0.41		0.459	0.247

TG: triglycerides; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol.
Continuous variable was showed as mean±SD.
†*p*-values was calculated by Student's paired t test to the changes from baseline to the end of intervention.
‡*p*-values value was calculated by a crude general linear model to test the difference in treatment effects over time between the two groups.
§*p*-values was calculated by a full general linear model with adjustments for BP, BMI, age, gender, and baseline values to test the difference in treatment effects over time between the two groups.

Table 3. Utility of treatments in the serum inflammatory factors

	B vitamins group (n=42)			*p*-values[1]	Control group (n=48)			*p*-values[1]	*p*-values[2]	*p*-values[3]
	Time				Time					
	Baseline	Endpoint	Change		Baseline	Endpoint	Change			
Hcy (μmol/L)	12.80±2.50			0.001	13.42±3.25			0.001	0.314	0.709
		9.78±2.04				11.88±2.85			<0.001	0.001
			3.02±2.35				1.55±1.58		<0.001	0.001
IL-1β (pg/mL)	71.49±13.36			0.001	72.53±14.50			<0.001	0.724	0.419
		57.16±14.31				66.59±13.49			0.001	0.008
			14.33±6.40				5.94±8.80		<0.001	0.001
TNF-α (ng/mL)	20.45±7.56			0.168	20.99±7.35			<0.001	0.734	0.501
		16.02±4.62				15.71±6.54			0.796	0.434
			4.43±7.95				5.28±5.53		0.554	0.908
IL-6 (pg/mL)	14.62±3.43			0.001	13.52±2.54			<0.001	0.083	0.152
		8.82±2.85				8.59±2.93			0.705	0.898
			5.79±1.74				4.93±3.61		0.157	0.110
IL-4 (ng/L)	33.71±5.08			0.002	32.50±7.78			0.64	0.238	0.259
		32.67±5.71				31.53±5.86			0.354	0.309
			1.05±5.65				0.52±7.65		0.713	0.837
CRP (mg/L)	1.33±0.99			0.001	1.50±1.23			<0.001	0.485	0.595
		0.82±0.83				0.78±0.88			0.869	0.227
			0.52±0.70				0.71±0.65		0.168	0.533
	71.49±13.36			0.936	23.84±3.82			<0.001	0.655	0.955
APN (ng/mL)		57.16±14.31				26.16±4.60			0.049	0.099
			-3.82±7.39				-2.31±6.46		0.302	0.274
IL-10 (ng/L)	16.68±3.02			<0.001	15.60±3.16			<0.001	0.098	0.146
		21.81±4.25				18.80±3.50			<0.001	<0.001
			-5.13±3.80				-3.20±4.13		0.022	0.016

Hcy: homocysteine; IL-6: interleukin-6; IL-1β: interleukin-1β; IL-4: interleukin-4; TNF-α: tumor necrosis factor-α; CRP: c-reactive protein; IL-10: interleukin-10; APN: adiponectin.
Continuous variable was showed as mean±SD.
[†]*p*-values was calculated by Student's paired t test to the changes from baseline to the end of intervention.
[‡]*p*-values value was calculated by a crude general linear model to test the difference in treatment effects over time between the two groups.
[§]*p*-values was calculated by a full general linear model with adjustments for BP, BMI, age, gender, and baseline values to test the difference.

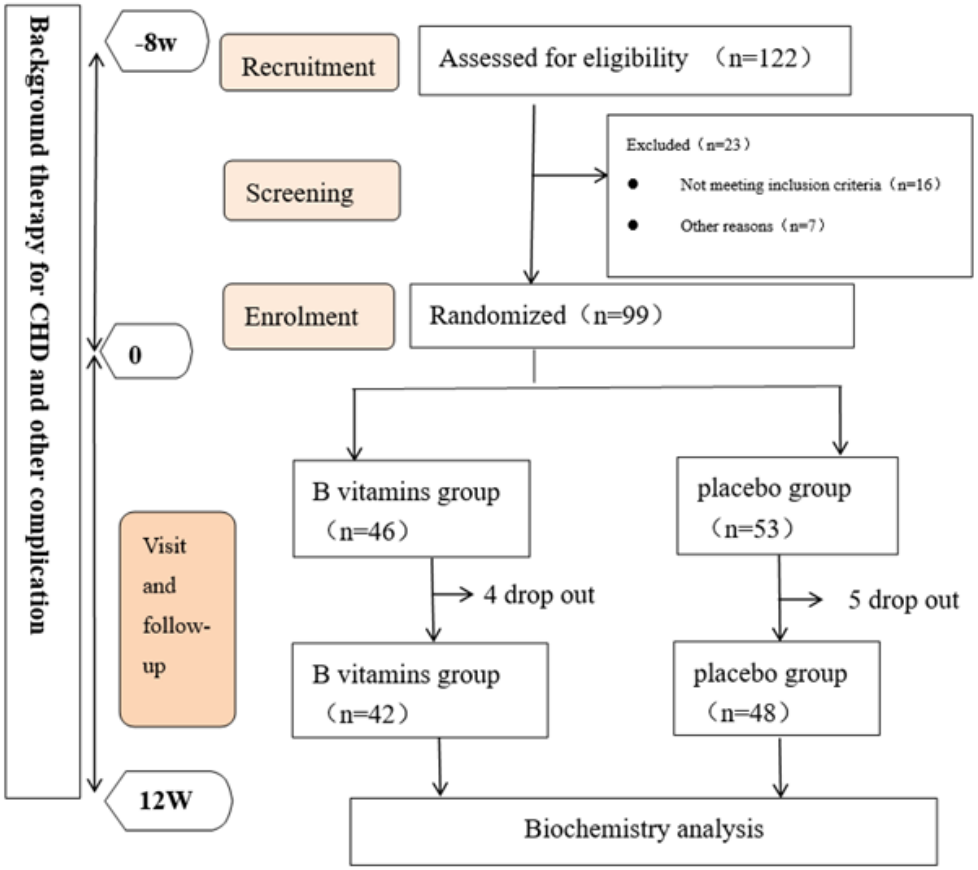

Figure 1. Flow chart of study participants