Original Article

Determination of Vitamin B12 Deficiency Status in Vegan and Non-Vegan Thais by Assessment of Homocysteine Level

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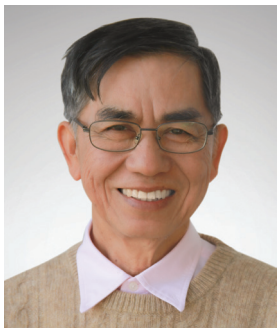

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Abstract

OBJECTIVES: To determine and compare vitamin B12 status in different food patterns by using serum homocysteine levels in two adult groups consisting of (1) one hundred participants in non-vegan group and (2) two hundred participants in vegan group. The vegan group is further divided into two subgroups (2.1) ordinary vegan who consumes vegan diets for more than three years and less than twenty years (2.2) high risk vegan who consumes vegan diets for more than twenty years or those who are over 65 years old or those who have signs or symptoms suggesting vitamin B12 deficiency.

MATERIAL AND METHODS: This study is a descriptive cross-sectional study designed to compare the status of vitamin B12 using homocysteine level in the total 300 participants in vegan and non-vegan Thais, performed in October 2018. The study used a chemiluminescent microparticle immunoassay (CMIA) using an Architec machine to determine blood homocysteine levels. Their levels of homocysteine were then compared. The data was analyzed using the unpaired *t*-test.

RESULT: Three hundred non-vegan and vegan Thais were included in this study. One hundred and five of these were male and 195 were female. Of these, 41% had high homocysteine levels (more than 15.4 Umol/L.), 59% of them had normal homocysteine levels (less than 15.4 Umol/L). Among one hundred non-vegans Thais (gr.1), there was only one (1%) who had a high homocysteine level. Among one hundred ordinary vegans (gr.2.1), fifty-two (52%) of them had abnormally high homocysteine. Among one hundred of high-risk vegans (gr.2.2), seventy of them (70%) had high homocysteine level. The vegan group had higher blood levels of homocysteine ($p < 0.001$) than the non-vegan group.

CONCLUSION: Vegan Thais have higher risk of vitamin B12 deficiency as determined by their abnormally higher homocysteine blood levels than that of non-vegans ($p < 0.001$). We recommend that all Thai vegans should take vitamin B12 supplements regularly to prevent vitamin B12 deficiency. The dosage recommended by the American Vegetarian Medical Association is 50-100 micrograms per day or 500-1,000 micrograms per week. Thai vegans who do not take B12 supplementation should be screened for B12 deficiency.

Keywords: vitamin B12 deficiency, homocysteine level, vegan , non-vegan

Vitamin B12 is a water-soluble nutrient containing the mineral cobalt, collectively called cobalamine. Only two forms of vitamin B12, methylcobalamin and 5-deoxyadenosylcobalamin, are active in the human metabolism. It is derived mainly from animal-source food[1] such as beef, clams, sardines, tuna, trout, salmon, poultry, eggs, milk and dairy products, fortified cereal, fortified nutritional yeast, fortified non-dairy milk, etc. The recommended daily intake (RDI) for vitamin B12 is 2.4 micrograms per day.

Once consumed, vitamin B12 is released from protein in food by hydrochloric acid and gastric protease in the stomach to become free B12. It then combines with a glycoprotein secreted by the parietal cell of the stomach, called intrinsic factor, to become a complex absorbed at the distal ileum. Vitamin B12 is reasonably well absorbed (56%) if taken orally at low dose (1 micrograms). At high doses, the absorption of vitamin B12 decreases drastically when the capacity of intrinsic factor is exceeded.

The body cannot absorb vitamin B12 in pernicious anemia. This is an autoimmune disease that affects the gastric mucosa and results in gastric atrophy leading to the destruction of parietal cells and failure to produce intrinsic factor. Pernicious anemia leads to vitamin B12 deficiency with subsequent megaloblastic anemia and neurological disorders despite adequate dietary intake of vitamin B12.

Once absorbed into plasma, the half-life of vitamin B12 in plasma, as documented by radioactive B12 tracing, is 5.14 days. The body can store excess vitamin B12 for future use in the liver for as long as 3-5 years.

Vitamin B12 is required for proper red blood cell formation, neurological function, and DNA synthesis. Metabolically it functions as a cofactor for methionine synthase which catalyzes the conversion of homocysteine to methionine (an amino acid to form S-adenosylmethionine which is a universal methyl donor for almost 100 different substrates, including DNA, RNA, hormones, proteins, and lipids). Vitamin B12 also functions as a cofactor for L-methylmalonyl-CoA mutase which converts L-methylmalonyl-CoA to succinyl-CoA, an essential requirement for fat and protein metabolism and hemoglobin synthesis.

There is evidence to support that those who are at risk of vitamin B12 deficiency can be categorized into the following three groups:

1. Children younger than eleven months who have not consumed animal-source foods.[2]
2. Elderly.[3]
3. Adult vegans who do not eat animal-based food at all.[4-11]

Research comparing the incidence of ischemic heart disease and the length of life among vegans in England has shown that there was no significant difference in the length of life between vegans and non-vegans even though vegans had lower plasma lipid levels, the main risk factor of cardiovascular disease.[12] An analysis of five studies with over 24,000 vegetarians also indicated that lacto-ovo vegetarians had a lower mortality rate from ischemic heart disease than strict vegans.[13] The vegans had a higher length of life than lacto-ovo vegetarians only when vegans regularly take vitamin B12 supplement.[14]

Vitamin B12 status is typically assessed via serum or plasma levels of vitamin B12. Values below approximately 170-250 pg/mL (120-180 picomols/L) for adults indicate a vitamin B12 deficiency. The direct measurement of vitamin B12 level is relatively insensitive (sensitivity 50%) due to the false-positive result creates by molecules of inactive vitamin B12 analogs. In addition to direct measurement, the accuracy of diagnosis of vitamin B12 deficiency can be improved by a combination of the following 3 methods.[15-18]

- ***Method 1:*** *Determination of serum homocysteine level.* Since the human body needs vitamin B12 to convert homocysteine back into methionine, vitamin B12 deficiency causes accumulation of homocysteine. Determination of serum homocysteine level has much better (95.6%) sensitivity in the detection of vitamin B12 deficiency. The flaw of this method is its low specificity because homocysteine can be elevated in deficiency of folate (B9) and pyridoxine (B6).
- ***Method 2:*** *Determination of serum methyl malonic acid (MMA) level.* Since in the normal human body, vitamin B12 helps transform MMA into an energy substrate called succinyl-CoA. Vitamin B12 deficiency leads to abnormally high level of MMA. This type of examination has a sensitivity of up to 98.6%.
- ***Method 3:*** *A combination of determining both homocysteine and MMA level.* This method has 99.8% sensitivity.

In this study we chose Method 1, determination of serum homocysteine level, considering:

1. Its financial affordability
2. The low specificity of this method is offset by the low incidence of folate and pyridoxine deficiency among vegetarians, who are the volunteer subjects of this study
3. The sensitivity 95.6% is acceptable.

In Thailand, so far, there have been no studies looking at homocysteine levels in the normal Thai population. There is indirect information from the study of homocysteine levels in Thai smokers,[19] in which the level of homocysteine was assessed in 174 Thai smokers and 97 non-smokers. The result showed that the median homocysteine level in non-smokers was 11.81 micromoles/liter, while in smokers it was 13.24 micromoles/liter. The universally accepted normal value of homocysteine is between 4 and 15.4 micromoles/liter.

The clinical manifestation of vitamin B12 deficiency can be categorized into three groups:

1. Neuropsychiatric disorders[20]
2. Megaloblastic anemia[21]
3. Cardiovascular diseases[22]

In this study, we divided subjects into two groups:

- gr.1 (non-vegans)
- gr.2 (vegans)

Since the ability to store vitamin B12 in the body is around 3-5 years, we recruited only those who had been vegans for not less than 3 years. Within the vegans (gr.2), we subdivided further to separate ordinary vegans (gr.2.1) from high-risk vegans (gr.2.2).

High-risk vegans were arbitrarily defined as those who

1. had been vegans for more than 20 years, or
2. were older than 65 years of age, or
3. had signs or symptoms related to vitamin B12 deficiency (anemia, neurologic symptoms, cardiovascular symptoms)

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In this study, we defined vegans as those who ate strictly non-animal-based food (no meat, no fish, no eggs, and no milk). The adherence to a vegan diet in the vegan group was ensured by recruiting only members of the strict religious vegan practice group of Santi Asoke Community into this study, and by rigorous interview of the volunteers.

Prevention of vitamin B12 deficiency is important, particularly in high-risk groups such as strict vegans and elders.

The purpose of this research is to answer the question that in order to maintain normal level of vitamin B12, should Thai vegans take vitamin B12 supplement?

Materials and Methods

This study is a descriptive, cross-sectional study designed to determine serum homocysteine levels in adult volunteers using the following methodology:

Step 1: Volunteer recruitment. The selection criteria include:

1. Age of 18 years and above
2. Has not been taking any vitamin supplements, particularly vitamin B12, vitamin B complex, and multivitamins.

The recruited were non-randomly divided into three groups based on their existing food pattern.

Group 1: Non-vegans. One hundred adults who consumed an ordinary Thai diet which included meat, poultry, milk, eggs, etc.

Group 2: Vegans. Two hundred adults who were members of the strict religious vegan practice "Santi Asoke Community", and had been vegans for more than 3 years, were recruited. They were subdivided into two subgroups:

Subgroup 2.1: Ordinary vegans. One hundred vegan adults aged 18 - 65 years who had followed a regular vegan diet for more than 3 years but less than 20 consecutive years.

Subgroup 2.2: High-risk vegans. One hundred vegan adults who met at least one of these 5 criteria:

1. Being vegan > 20 years
2. Age > 65 years
3. Had megaloblastic anemia
4. Had neuropsychiatric disorders
5. Had cardiovascular diseases

Step 2: Interviewing nutritional history and adherence to vegan diet.

Step 3: Physical examination to detect anemia, neuropsychiatric disorders, and cardiovascular disorders.

Step 4: Taking of blood samples to determine serum homocysteine levels by CMIA method using Architech machine from the Abbot Company.

Step 5: Statistical analysis of the relation between being vegan and non-vegan versus vitamin B12 status by comparing the homocysteine levels between the two groups using unpaired t-test, accepting a value of $p < 0.01$.

Results

Part 1: Homocysteine status of the samples

In the one hundred non-vegan group subjects (gr.1), the range of serum homocysteine levels was 3.5-43.5 micromoles/liter. The average value was 8.13 micromoles/liter. One subject (1%) had abnormally high homocysteine. Ninety-nine (99%) had normal homocysteine levels.

In the one hundred ordinary vegan group subjects (gr.2.1) who had been vegans for 3-20 consecutive years, the range of plasma homocysteine level was 5.6-80.5 micromoles/liter. The average value was 18.1 micromoles/liter. Forty-eight subjects (48%) were within normal range. Fifty-two (52%) had abnormally high homocysteine levels.

In the one hundred high-risk vegan group subjects (gr.2.2) who had been vegans for more than twenty consecutive years, were older than 65, or had clinically suggestive manifestations of vitamin B12 deficiency, the average value was 19.9 micromoles/liter. Thirty (30%) had normal homocysteine levels. Seventy (70%) had abnormally high homocysteine levels.

Part 2: Comparison of homocysteine level between vegan and non-vegan groups

Analysis of homocysteine levels between the vegan and non-vegan groups using the unpaired t-test showed a significant difference between the two groups, with $p < 0.001$, as shown in Table 1.

Table 1 : Comparison of homocysteine levels between vegan and non-vegan groups

Group	n	Homocysteine level					
		Minimum	Maximum	Mean	Std. Deviation	*t*	*p*
Group 1: non-vegan	100	3.5	43.5	8.13	4.32	-7.820	< 0.001
Group 2: total vegan	200	5.6	141.5	18	12.3		
Subgroup 2.1: low-risk vegan	100	5.6	80.5	18.10	10.16		
Subgroup 2.2: high-risk vegan	100	7.9	141.5	19.90	14.26		

Discussion

In this study, we chose to determine vitamin B12 deficiency via homocysteine levels instead of using a combination of direct measurement of vitamin B12, homocysteine, and MMA levels, considering (1) its financial affordability, (2) the method's low specificity being offset by the low incidence of folate and pyridoxine deficiency among vegetarians who are volunteer subjects of this study, and (3) the sensitivity of 95.6% is comfortably acceptable for the purpose of this study.

Grouping of volunteer subjects in this study was done non-randomly, based on their existing food patterns. We roughly divided them into two groups, non-vegans and vegans. The adherence to vegan diet of the vegan group was ensured by recruiting only members of the strict religious vegan practice "Santi Asoke Community" into the group and by rigorous interviews to screen out those who were not completely adherent to the vegan diet. The method of study was a cross-sectional epidemiology study, not an interventional study. Still, the obtained results ($p < 0.001$) strongly indicate the relation between vegan food pattern and vitamin B12 deficiency. Since food pattern is a way of life and not the kind of factor suitable for randomized controlled studies, we suggest that no further study is required. The result of this study is enough to support the provision of vitamin B12 supplements among Thai vegans.

Within vegans, those who were defined as high-risk vegans related even more with vitamin B12 deficiency. The so-called high risk vegans included those who had been on a vegan diet for quite some time (more than 20 years), or those who were elderly (more than 65 years old), or those who had at least one suggestive clinical manifestation of vitamin B12 deficiency (anemia, neuropsychiatric disorders and cardiovascular disease). So it is sensible to emphasize vitamin B12 supplementation in these high-risk vegans.

Conclusion

The incidence of vitamin B12 deficiency as determined by homocysteine level is very low (1%) among 100 subjects from a Thai community population who consumed a regular diet. On the other hand, the incidence of vitamin B12 deficiency in ordinary vegans is very high (52%) and even higher (70%) in high-risk vegans who had been vegans for more than 20 years, or were older than 65 years, or had suggestive clinical manifestations of vitamin B12 deficiency (anemia, or neuropsychiatric disorders, or cardiovascular disorders). The markedly lower levels of vitamin B12 among Thai vegans is so striking ($p < 0.001$) that oral supplement of vitamin B12 is sensible. Hence, we recommend that all strict Thai vegans should take vitamin B12 supplementation regularly. The dosage recommended by the American Vegetarian Medical Association is 50-100 micrograms per day or 500-1,000 micrograms per week. Those who are reluctant to take B12 supplements should be screened for vitamin B12 deficiency.

Conflict of interest statement

This study has been supported by (1) A research grant from Dr. Chawin Thammanunkun Foundation. (2) Muaklek Hospital, a community hospital in Saraburi Province, Thailand. (3) Rajathani Asoke, a vegan community in Ubolrajathani Province, Thailand. All these three entities have no conflict of interest with this study. All the authors also claim no conflict of interest associated with the manuscript.

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References

1. Allen LH. Causes of vitamin B12 and folate deficiency. *Food Nutr Bull* 2008;29 (Suppl 1):S20-S34.
2. Center for Disease Control and Prevention (CDC). Vitamin B12 Deficiency. (Accessed at July10, 2018, at http://www.cdc.gov/ncbddd/b12/table6.html)
3. Pennypacker LC, Allen RH, Kelly JP, et al. High prevalence of cobalamin deficiency in elderly outpatients. *J Am Geriatr Soc* 1992;40:1197-204.
4. Majchrzak D, Singer I, Manner M, et al. B-vitamin status and concentrations of homocysteine in Austrian omnivores, vegetarians and vegans. *Ann Nutr Metab* 2006;50:485-91.
5. Bissoli L, di Francesco V, Ballarin A, et al. Effect of vegetarian diet on homocysteine levels. *Ann Nutr Metab* 2002;46:73-9. doi: 10.1159/000057644.
6. Koebnick C, Hoffmann I, Dagnelie PC, et al. Long-term ovo-lactovegetarian diet impairs vitamin B-12 status in pregnant women. *J Nutr* 2004;134:3319-26.
7. Hokin BD, Butler T. Cyanocobalamin (vitamin B-12) status in the seventh-day adventist ministers in Australia. *Am J Clin Nutr* 1999;70(Suppl. 3):576S-578S. doi: 10.1001/jamainternmed.2013.6473.
8. Oberley MJ, Yang DT. Laboratory testing for cobalamin deficiency in megaloblastic anemia. *Am J Hematol* 2013;88:522.
9. Amos R, Dawson D, Fish D, et al. Guidelines on the investigation and diagnosis of cobalamin and folate deficiencies. A publication of the British Committee for Standards in Haematology. BCSH General Haematology Test Force. *Clin Lab Haematol* 1994;16(2):101-15.
10. Savage DG, Lindenbaum J, Stabler SP, et al. Sensitivity of serum methylmalonic acid and total homocysteine derterminations for diagnosing cobalamin and folate deficiencies. *Am J Med* 1994; 96:239-46.

11. Lindenbaum J, Savage DG, Stabler SP, et al. Diagnosis of cobalamin deficiency: II. Relative sensitivities of serum cobalamin, methylmalonic acid, and total homocysteine concentrations. *Am J Hematol* 1990; 34:99-107.
12. Key TJ, Appleby PN, Davey GK, et al. Mortality in British vegetarians: review and preliminary results from EPIC-Oxford. *Am J Clin Nutr* 2003 Sep; 78(3 Suppl):533S-538S.
13. Key TJ, Fraser GE, Thorogood M, et al. Mortality in vegetarians and nonvegetarians: detailed findings from a collaborative analysis of 5 prospective studies. *Am J Clin Nutr* 1999;70(3 Suppl):516S-524S.
14. Orlich MJ, MD, Singh PN et. al, Vegetarian Dietary Patterns and Mortality in Adventist Health Study 2. *JAMA Intern Med* 2013;173(13):1230-8. doi: 10.1001/jamainternmed.2013.6473
15. Oberley MJ, Yang DT. Laboratory testing for cobalamin deficiency in megaloblastic anemia. *Am J Hematol* 2013;88:522.
16. Amos R, Dawson D, Fish D, et al. Guidelines on the investigation and diagnosis of cobalamin and folate deficiencies. A publication of the British Committee for Standards in Haematology. BCSH General Haematology Test Force. *Clin Lab Haematol* 1994;16(2):101-15.
17. Savage DG, Lindenbaum J, Stabler SP, et al Sensitivity of serum methylmalonic acid and total homocysteine derterminations for diagnosing cobalamin and folate deficiencies. *Am J Med* 1994; 96:239-46.
18. Lindenbaum J, Savage DG, Stabler SP, et al. Diagnosis of cobalamin deficiency: II. Relative sensitivities of serum cobalamin, methylmalonic acid, and total homocysteine concentrations. *Am J Hematol* 1990;34:99–107.
19. Suriyaprom K, Tungtrongchitr R, Pongpaew P, et al. Hmocysteine and vitamin status in healthy Thai smokers. *Environ l Med* 2005;15(1): 9-21.
20. Lindenbaum J, Healton EB, Savage DG, et al. Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. *N Engl J Med*. 1988; 318:1720–8.
21. Zittoun J. Anemias due to disorder of folate, vitamin B12 and transcobalamin metabolism. *Rev Prat* 1993; 43(11):1358-63.
22. Wilcken DE1, Wilcken B. B vitamins and homocysteine in cardiovascular disease and aging. *Ann N Y Acad Sci* 1998;854:361-70.