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Research Article

Cobalamin and Methylmalonic Acid as Biomarkers of Vitamin B12 Deficiency in Elderly

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ABSTRACT
Cobalamin as surrogate biomarker of vitamin B12 has various limitations. There are several other markers that can be used to determine the vitamin B12 concentration in the body, such as methylmalonic acid (MMA). The objective of this study was to determine the prevalence of vitamin B12 deficiency using appropriate cut-off levels of cobalamin and MMA and to determine the relationship between cobalamin and MMA as biomarkers of vitamin B12 deficiency in the elderly. This study was of cross-sectional design and was performed on 80 elderly women aged 60-75 years, from January to April 2019. All subjects were asked to fill in a questionnaire, then the subjects meeting the inclusion and exclusion criteria underwent examination for anthropometric measures, vital signs, dietary recall, food frequency for vitamin B12, serum MMA and cobalamin. Data analysis was carried out with inter-quartile one-way Anova and the Spearman test between MMA and cobalamin concentrations, at level of significance p of < 0.05. The prevalence of B12 deficiency based on cobalamin was 8.75%, whereas the prevalence based on MMA was 72.3 %. The MMA concentration did not decrease consistently with increasing inter-quartile cobalamin concentration. The results of the Spearman test showed a low negative correlation between cobalamin and MMA, with r = -0.233 and p<0.043. In conclusion, the prevalence of B12 deficiency based on the MMA concentration was higher than that based on the cobalamin concentration. There was a weak negative correlation between cobalamin and MMA concentrations.

Keywords: Methylmalonic acid, MMA, cobalamin, elderly, vitamin B12 deficiency

INTRODUCTION

Vitamin B12 (cobalamin) deficiency is a frequently found condition in the elderly, that tends to increase with increasing age [1,2]. Cobalamin is an essential vitamin that is required by the body and is used in various enzymatic processes throughout the body. The main function of cobalamin is to synthesize deoxyribonucleic acid (DNA) in the bone marrow and to maintain myelin in both the peripheral and the central nervous system [1]. If undetected, vitamin B12 deficiency may result in reversible neurocognitive abnormalities [2]. The prevalence of vitamin B12 deficiency in the elderly ranges between 5% and 40%, depending on the definition of vitamin B12 deficiency that is used [3,4,5]. Severe vitamin B12 deficiency rarely causes difficulties in diagnosis; however, it is the initial stage of the deficiency that is difficult to diagnose [3]. This is due to the fact that almost 50% of patients with the subclinical condition has a normal cobalamin concentration [6]. Since the signs and symptoms of vitamin B12 deficiency are frequently not encountered at the onset of the deficiency, special attention should be paid especially in persons known to be at high risk of this condition [3]. The causes of vitamin B12 deficiency are categorized into three groups, namely abnormal gastrointestinal absorption, inadequate nutrient intake such as in vegans and elderly persons, and increased requirements for vitamin B12 such as in hemolysis and leukemia. Among the above-mentioned factors, the main cause of vitamin B12 deficiency is inadequate intake of foods of animal origin, such as meats and eggs [1].

Currently, the concentration cut-off point recommended by the WHO is <140 pmol/L (200 pg/mL). The WHO has used this cut-off point for the diagnosis of vitamin B12 deficiency since 2008 [7]. Up to now there is no consensus or guideline on the cut-off point for the diagnosis of vitamin B12 deficiency [8,9,10]. The diagnosis of vitamin B12 deficiency is usually established on the basis of clinical symptoms with additional laboratory results such as a low cobalamin

concentration and increased homocysteine or methylmalonic acid (MMA) concentration [8,11]. If vitamin B12 deficiency is suspected on clinical grounds, the laboratory examination requested is usually the cobalamin concentration, full blood count, and peripheral blood morphology. The latter will yield the classical picture of megaloblastic anemia, but frequently this condition is not seen, especially in moderate anemia [11,12]. Determination of the cobalamin concentration is usually recommended if a patient with macrocytosis is found, but macrocytosis with or without anemia is not sensitive and not specific for establishing the diagnosis of vitamin B12 deficiency. This is because macrocytosis may be found in other conditions, such as folate deficiency and the myelodysplastic syndrome, and more than 84% of cases will not be diagnosed if macrocytosis is the only basis for screening for vitamin B12 deficiency [8,13,14].

Determination of the concentration of vitamin B12 can at present be performed and is relatively inexpensive [15]. What need to be considered are the limitations of using cobalamin for the diagnosis of vitamin B12 deficiency, such as the inconsistency between serum cobalamin concentration and clinical vitamin B12 deficiency, so that more sensitive screening methods for vitamin B12 deficiency are needed. One of them is by measuring vitamin B12 metabolites that accumulate as a result of vitamin B12 deficiency, among others the MMA concentration [6,15]. Methylmalonic acid is the end-product of the breakdown of amino acids into methylmalonyl CoA in the citric acid cycle [1]. Vitamin B12 is required for the conversion of MMA into succinyl coenzyme A. The presence of vitamin B12 deficiency in the tissues will increase the MMA concentration both in plasma and the urine, even though the serum cobalamin concentration is within the normal range [6,14]. The gold standard for determining vitamin B12 deficiency is an increased MMA concentration [8]. The MMA concentration increases with decreasing vitamin B12 concentration, but the MMA concentration may also increase spuriously in subjects with renal disorders, bacterial overgrowth in the small intestine, and hemoconcentration. Even with these limitations, an increased MMA concentration nearly always indicates vitamin B12 deficiency [11]. The use of MMA for determining functional vitamin B12 deficiency is still infrequent, particularly in the elderly. The aim of this study was to determine the prevalence of vitamin B12 deficiency by using the cut-off levels of cobalamin and MMA and by comparing cobalamin and MMA as biomarkers of vitamin B12 deficiency in the elderly.

METHODOLOGY

Research Design and Study Subjects

This study of cross-sectional design was performed from January 2019 to April 2019. The inclusion criteria were elderly females aged between 60 and 75 years who resided in Mampang Prapatan District, South Jakarta, agreeing to participate in this study (by signing informed consent), not suffering from stroke and other terminal diseases such as malignancies, diabetes mellitus, renal failure, and myocardial infarction. The exclusion criteria in this study were use of corticosteroids, hormonal replacement therapy, and vitamin B12 and folate supplements.

Questionnaire and Measurements

One hundred elderly females meeting the inclusion and exclusion criteria were interviewed by four interviewers (with minimally senior high school education or equivalent) who had received prior training on the aim of the study and the filling-out of the questionnaire. The questionnaire comprised the subject's name, age, address, diseases suffered, supplement and medications taken. The study subjects meeting the inclusion and exclusion criteria were asked to fast for minimally 12 hours prior to blood collection for laboratory examination. They also underwent physical examination comprising blood pressure using a non-mercury sphygmomanometer/digital sphygmomanometer, pulse rate using a stopwatch, temperature, weight, height, hip circumference and abdominal circumference.

Laboratory Examinations

From each of the subjects a volume of 10 mL venous blood was drawn, to be made into serum for creatinine measurement and the rest stored at minus 20^{0}C for simultaneous examination of cobalamin and MMA concentrations. The examination of cobalamin used the fluorescent polarization immunoassay, whereas the MMA examination used the enzyme-linked immunosorbent assay (ELISA). The cobalamin concentration is said to indicate B12 deficiency if it is < 150 pmol/L (200 pg/mL) and the MMA concentration is said to indicate B12 deficiency if it is > 210 nmol/L [7].

Food Frequency Questionnaire

To determine the daily food intake of the study subjects, particularly of vitamin B12-containing foods, dietary recall was carried out on 2 working days and 1 Saturday/Sunday or holiday and interviews using a food frequency questionnaire to determine the intake of vitamin B12-containing foods were performed by 2 trained enumerators (nutritional personnel).

Statistical Analysis

The data were analyzed using Statistical Package for Social Science Students (SPSS) software version 21. Normally distributed data were presented as mean ± standard deviation (SD), whereas non-normally distributed data were presented as median and minimum-maximum range. To find a correlation between cobalamin and MMA concentration, the Pearson correlation test was used for normally distributed data and the Spearman Rho for non-normally distributed data, a value of $p < 0.05$ indicating significant differences. The inter-quartile one-way Anova was performed to analyze MMA concentration based on quartiles of serum cobalamin with p value < 0.05.

Ethical Clearance

Informed consent was obtained after the subjects clearly understood the goal of this study and were willing to participate. This study obtained ethical clearance from the Faculty of Medicine, Trisakti University, under no. 136/KER/FK/I/2019.

RESULTS

Of the 100 subjects who agreed to participate, there were 20 subjects who were rejected, because among them 10 persons were under 60 years of age and the other 10 persons were absent during the study. The mean age of the subjects was 65.4 ± 4.2 years. The most frequent educational status of the subjects was elementary school graduate in 33.8% and senior high school graduate in 33.8%. The majority of the subjects were retired (80%). The anthropometric data and vital signs are shown in Table 1. For the cobalamin (vitamin B12) concentration, the median was 255.6 pmol/L with lower and higher inter-quartile ranges of 121.5 and 1446.7 pmol/L, respectively. Most of the subjects (73 or 91.25 %) had a B12 concentration of ≥ 150 pmol/L or belonged to the normal category. Based on MMA concentration, the number of subjects with a normal vitamin B12 concentration was only 22 (27.7 %) (Table 1).

Characteristics of the Study Subjects

Table 1: Characteristics of the Study Subjects

Characteristic	X ± SD (n=80)
Age (years)	65.4 ± 4.2
< 65----n (%)	42 (52.5)
≥ 65 ---n (%)	38 (47.5)
Gender	
Female ---n (%)	80(100)
Education	
No schooling	5 (6.3)
Elementary school---n (%)	27 (33.8)
Junior high school---n (%)	17 (21.3)
Senior high school ---n (%)	27(33.8)
Academy ---n (%)	4 (5)
Employment status	
Employed ----n (%)	16 (20)
Unemployed ---n (%)	64 (80)
Ethnicity	
Javanese-------n (%)	46 (58.1)
Sundanese------n (%)	9 (12)
Betawi-----n (%)	4 (4.3)
Padang-----n (%)	17 (21.3)
Other---n (%)	4 (4.3)
Anthropometrics	
Height (cm)	152.3 ± 6.6
Weight (kg)	59.1 ± 9.6
BMI (kg/m^2)	25.5 ± 3.8
Abdominal circumference (cm)	87.3 ± 7.8
Hip circumference (cm)	103.4 ± 8.2
Vital signs	
Pulse rate (/minute)	79.8 ± 5.1

Systolic blood pressure (mmHg)	126.1 ± 20.6
Diastolic blood pressure (mmHg)	80.3 ± 12.6
Biochemistry	
Creatinine (mg/dL)	0.84 ± 0.19
eLFG	76.4 ± 17.8
B12 (pmol/L)----median (min-max)	255.6 (121.5 – 1446.7)
< 150 (deficiency)----n (%)	7 (8.75)
≥ 150 (normal)-------n (%)	73 (91.25)
MMA (nmol/L)	252.0 ± 65.9
>210 (deficiency)-----n (%)	58 (72.3)
≤ 210 (normal)-------n (%)	22 (27.7)

Table 2: Serum MMA Concentration Based on Quartiles of Serum Cobalamin

Serum cobalamin (pmol/L)				
	Q1 (≤ 201)	Q2 (202 - (≤ 298)	Q3 (299 - (≤ 511)	Q4 (> 512)
n(%)	21 (26.3)	21 (26.3)	18 (22.4)	20 (25)
MMA (nmol/L)[2]	263.9 ± 57.5	277.5 ± 57.5	221.7 ± 78.7[3]	239.4 ± 60.9

Data are expressed as mean ± SD
[2]Significant inter-quartile differences (one way Anova) p=0.043
[3]Significant difference with Q1 (LSD post hoc test) p=0.047

In Table 2 are presented the mean and standard deviation of MMA inter-quartile concentrations by serum cobalamin concentration. The MMA concentration did not decrease consistently with increasing cobalamin concentration. The results of interquartile one-way Anova showed a significant difference at p=0.043. The significant difference was in the third quartile as compared with the first quartile at p=0.047.

Table 3: Serum MMA Concentration Based on Quartiles of Serum Cobalamin

Nutrient intake	X ± SD n=80
Energy (kcal)	1206.1±354.9
Carbohydrate (g)	156.9 ± 51.8
Protein (g)	40.5 ± 13.3
Fat (g)	44.7 ± 17.3
Vitamin B12 (µg)	1.93 ± 0.2
≤ 2.4 µg (n/%)	64 (79.7)
>2.4 µg (n/%)	16 (20.3)

Table 3 shows the intakes of macro- and micronutrients of the study subjects. The majority of the subjects had a vitamin B12 intake of < 2.4 µg, namely 64 subjects (79.7%). Mean vitamin B12 intake of the subjects was 1.93 ± 0.2 µg.

Table 4: Possible Correlations between a Numbers of Variables in the Study Subjects

Variable	Correlation coefficient (r)	p value
Age		
B12	0.127	0.274
MMA	-0.100	0.932
B12		
MMA	-0.233	0.043*

Spearman correlation test, p< 0.05 significant difference

Table 4 shows that there were no significant correlations of age with vitamin B12 and MMA. From the results of the Spearman correlation test, for cobalamin and MMA the resulting r was -0.233 at p=0.043, indicating that there was a low correlation between them.

DISCUSSION

Vitamin B12 deficiency is a frequently encountered condition in the elderly population[1,16]. In the present study it was found that the prevalence of vitamin B12 deficiency based on a cobalamin concentration of < 150 pmol/L was 8.75 % of the elderly females (Table 1). The definition of vitamin B12 deficiency in this study was a cobalamin concentration of < 150 pmol/L, in accordance with the cut-off point used by the WHO since 2008 [7,17]. This cut-off point is the most frequently used for the diagnosis of vitamin B12 deficiency [18,19]. The prevalence found in the present study agreed with the supposition that the use of this cut-off point would result in a prevalence of vitamin B12 deficiency of around 5-15 % [18]. The prevalence of vitamin B12 deficiency obtained in the present study was higher than the results of a US survey with a prevalence at age 40-59 years of around 4 % and at age > 70 years of around 6% [8]. The difference may have been due to the fact that the US is a developed country, whereas Indonesia is still in the category of developing countries. As stated by Allen, vitamin B12 deficiency in developed countries is frequently found, but is more common in developing countries [8]. The study results of Khodabandehloo showed a prevalence of vitamin B12 deficiency of 22.3 % in elderly females with mean age of 73.7± 5.7 years [20]. Vitamin B12 deficiency increases with increasing age [21]. Epidemiologic data show that the prevalence of vitamin B12 deficiency ranges between 6% and 40% [22]. The prevalence of vitamin B12 deficiency increases after the age of 69 years in a survey conducted in England, affecting one in 20 persons at age 65-74 years and at least one in 10 persons at age over 75 years [23,24].

The median vitamin B12 concentration in the present study was 255.6 pmol/L. The study of Khodabandehloo et al. reported a mean B12 concentration of 295 ± 170 pmol/L in their study subjects[20], whereas the study of Wolters et al. in Germany showed that the mean serum cobalamin was 290 ± 98.1 pmol/L[25]. The difference in cobalamin concentration between the present study and the other two may have been due to the fact that the intake of cobalamin-containing foods was lower in the subjects of the present study, as supported by the results of the food frequency questionnaire interview, showing that the mean vitamin B12 intake of the subjects was 1.93 ± 0.2 µg (Table 3). This value is lower than the Indonesian Recommended Daily Allowances (IRDA) of 2.4 µg as recommended by the Indonesian Department of Health (Depkes)[26]. The most common cause of vitamin B deficiency in menopause, including vitamin B12, is an inadequate intake. This may be due to increased requirements in menopausal women, to malabsorbtion caused by intestinal disorders, to diseases requiring medicines that interact with vitamin B metabolism, and to alcohol abuse, all significantly affecting the concentration of all members of the vitamin B group[27,28].

Based on an MMA concentration of > 210 nmol/L, the prevalence of vitamin B12 deficiency in the present study was found to reach 72.3 % with mean MMA concentration of 252 ± 65.9 nmol/L (Table 1). This indicates that the MMA concentration was already increased in elderly with a vitamin B12 concentration that was still within the low normal category (not yet < 150 pmol/L) so that if only the cobalamin examination is used, the condition of a functional vitamin B12 deficiency may not be detected. Cobalamin assay is recognized to be potentially unreliable because serum cobalamin measures cobalamin bound to the 2 circulating binding proteins, haptocorrin and transcobalamin (TC), and it is only the approximately 20%-30% of cobalamin bound to TC (i.e., holotranscobalamin) for which there is a receptor-mediated cellular uptake [9,15,29]. The function of haptocorrin is currently unknown, and low haptocorrin concentration, found in approximately 15% of persons with low serum cobalamin, could be one of the most common causes of low cobalamin concentrations [17]. Therefore, there is a need for the MMA examination, MMA being a metabolite that accumulates in the body when there is vitamin B12 deficiency, particularly in individuals with low-normal cobalamin concentrations (150-221 pmol/L) [2]. Methylmalonic acid is the gold standard indicator for determining vitamin B12 deficiency with a cut-off point of > 210 nmol/L in patients with normal renal function [8,15]. Serum MMA has a sensitivity of 98.4 % in diagnosing vitamin B12 deficiency [30]. The study of Carmel et al. who used the same MMA cut-off point as the present study, reported an increase in MMA concentration in 55.1 % of the elderly [24]. The study of Wolters et al. in elderly females with mean age of 63.2 ± 2.73 years found only 10 % of subjects with increased MMA concentration. The difference with the present study lies in the fact that in Wolters' study an MMA cut-off point of > 271 nmol/L was used [25]. The differences in cut-off levels for determining vitamin B12 deficiency

results in differences in prevalence that are encountered in the studies on vitamin B12. In addition, the use of MMA only for establishing the diagnosis of vitamin B12 deficiency may lead to overdiagnosis. Based on the above-mentioned results the use of MMA is recommended in subjects with low normal B12 or with symptoms of vitamin B12 deficiency but with normal blood cobalamin concentration [25]. What may have caused the difference in prevalence between these studies is the possibility of alcohol intake in the study of Wolters influencing vitamin B12 absorption [25], whereas in the study by Khodabandehloo there are no data on vitamin B12 intake[20]. The main causes of cobalamin deficiency in the elderly may be categorized into two groups, i.e. inadequate dietary intake and abnormalities of vitamin B12 absorption, such as in pernicious anemia, gastric atrophy, post-gastrectomy, and ileal resection [20,22]. Although MMA is more sensitive and specific than cobalamin, there is a need for a cautious interpretation of the MMA concentration especially in elderly with decreased renal function[1].

Table 2 shows that the MMA concentration does not decrease consistently with the increase in cobalamin concentration. This is also supported by the fact that there is a significant inter-quartile difference (one-way Anova) with $p<0.043$. The results of post-hoc analysis show that the difference lies between the MMA concentration in the first quartile and that of the third quartile, whereas the other quartiles do not show a difference. These study results are in line with the study results of Wolters et al. in that MMA concentration does not decrease consistently with increasing cobalamin concentration, at $p=0.001$, with post-hoc results also showing differences between quartiles 2 and 4 [25]. These results agree with the results of the correlation analysis, showing a low correlation between MMA and cobalamin concentrations.

Table 4 shows that there is no relationship of age with vitamin B12 as well as with MMA concentration. This differs from the results of the study by Khodabandehloo et al. who state that there is a negative correlation between B12 and age, with $r = -0.337$ and $p=0.001$. This may have been due to the fact that the age of the recruited subjects in this study ranged from 60 to 75 years, whereas in the study of Khodabandehloo et al. the mean age of the subjects was 73.7 ± 5.2 years. As is well-known, with increasing age there is also a decrease in renal function. In the present study the mean creatinine concentration of the study subjects was 0.84 ± 0.19 mg/dL, whereas in the study of Khodabandehloo et al. the mean creatinine concentration in males and females was 1.7 ± 0.3 mg/dL and 1.4 ± 0.3 mg/dL, respectively [20]. The decrease in renal function results in an increase in MMA concentration. Between cobalamin and MMA a low negative correlation was found in the present study, with $r = -0.233$ and $p=0.043$. This is in line with the study of Wolters et al. who showed a significant negative correlation with $r = -0.25$ and $p=0.001$ [25]. This agrees with the prediction that MMA is the metabolite that increases in vitamin B12 deficiency.

The limitation of this study lies in the fact that the subjects were exclusively elderly females and did not comprise still older persons. There is a need for early detection of vitamin B12 deficiency in the elderly using other vitamin B12 biomarkers, in addition to cobalamin, particularly in subjects with low-normal cobalamin concentrations or in subjects with clinical symptoms of vitamin B12 deficiency but whose cobalamin concentration is still within the normal range. There is also a need for input to Indonesian food policy makers for the fortification of foods with vitamin B12 so as to ensure an adequate intake of vitamin B12 in accordance with the recommendations in the Indonesian RDA.

CONCLUSION

In conclusion, the results of the present study indicate that the prevalence of vitamin B12 deficiency based on cobalamin concentration is lower than that based on MMA concentration (8.75% vs 72.3%). This may lead to misdiagnosis or delay of treatment to the patient because of misleadingly normal cobalamin results. MMA is a more sensitive biomarker than cobalamin for determining functional vitamin B12 deficiency in elderly females, but it should be ascertained that the subjects have normal renal function.

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REFERENCES

1. Carr F, Alagiakrishnan K. Challenges with the diagnosis of vitamin B12 deficiency in older adults. CGS journal of CME 2015;5:41-8.
2. Solomon LR. Low cobalamin levels as predictors of cobalamin deficiency: Importance of comorbidities associated with increased

oxidative stress. Am J Med 2016;129:115.e9-16. Doi:http://dx.doi.org/10.1016/j.amjmed.2015.07.017.
3. Loikas S, Koskinen P, Irjala K, Löppönen M, Isoaho R, Kivelä S, et al. Vitamin B12 deficiency in the aged: A population-based study. Age Ageing 2007;36:177-83. Doi:10.1093/ageing/afl150.
4. NICE 2015. Active B12 assay for diagnosing vitamin B12 deficiency. 2015. Nice.org.uk/guidance/mib40.
5. Langan RC, Zawistoski KJ. Update on vitamin B12 deficiency. Am Fam Physician 2011;83(12):1425-30.
6. Vashi P, Edwin P, Popiel B, Lammersfeld C, Gupta D. Methylmalonic acid and homocysteine as indicators of vitamin B12 deficiency in cancer. Plos One 2016;11(1):e0147843. Doi:10.1371/journal.pone.0147843.
7. De Benoist B. Conclusions of a WHO technical consultation on folate and vitamin B12 deficiencies. Food Nutr Bull 2008;29(2):S238-44.
8. Allen LH. How common is vitamin B12 deficiency. Am J Clin Nutr 2009;89:693S-6S.
9. Valente E, Scott JM, Ueland PM, Curningham C, Casey M, Molloy AM. Diagnostic accuracy of holotranscobalamin, methylmalonic acid, serum cobalamin, and other indicators of tissue vitamin B12 status in the elderly. Clin Chem 2011;57(6):856-63.
10. Obeid R, Heil SG, Verhoeven MMA, van den Heuvel EGHM, de Groot LCPGM, Eussen SJPM. Vitamin B12 intake from animal foods, biomarkers, and health aspects. Front Nutr 2019;6;93. doi:10.3389/fnut.2019.00093.
11. Devalia V, Hamilton MS, Molloy AM. Guidelines for the diagnosis and treatment of cobalamin and folate disorders. British J Hematol 2014;166:496-513.
12. Ankar A, Kumar A. Vitamin B 12 deficiency (cobalamin). NCBI bookshelf. StatPearls Publishing 2019.
13. Adaikalakoteswan A, Jayashri R, Sukumar N, Venkataraman H, Pradeepa R, Gokulakrishnan H, et al. Vitamin B12 deficiency is associated with adverse lipid profile in Europeans and Indians with type 2 diabetes. Cardiovasc Diabetol 2014;13:129.
14. Obeid R. Methylmalonic acid: A biomarker for vitamin B12 deficiency. Dialog 2014;1:1-3.
15. Hannibal L, Lysne V, Bjorke-Monsen AL, Behringer S, Grünert SC, Spiekerkoetter U, et al. Biomarkers and algorithms for the diagnosis of vitamin B12 deficiency. Front Mol Biosci 2016;3:27. Doi: 10.3389/fmolb.2016.00027.
16. Porter K, Hoey L, Hughes CF, Ward M, McNulty H. Causes, consequences and public health implications of low vitamin status in ageing. Nutrients 2016;8:725. doi:10.3390/nu8110725.
17. Carmel R. Mild transcobalamin I (haptocorrin) deficiency and low serum cobalamin concentrations. Clin Chem 2003;49:1367–74.
18. Wong CW. Vitamin B12 deficiency in the elderly: Is it worth screening? Hong Kong Med J 2015;21:155-64. DOI: 10.12809/hkmj144383.
19. Aparicio-Ugarriza R, Palacios G, Alder M, Gonzales-Gross M. A review of the cut-off points for the diagnosis of vitamin B12 deficiency in the general population. Clin Chem Lab Med 2015;53(8):1149-59. DOI: 10.1515/cclm-2014.0784.
20. Khodabandehloo N, Vakili M, Hashemian Z, Zardini HZ. Determining functional vitamin B12 deficiency in the elderly. Iran Red Crescent Med J 2015;17:e13138. DOI:10.5812/ircmj.17(6)2015.13138
21. Palacios G, Sola R, Barrios L, Pietrzik K, Castillo MJ, González-Gross M. Algorithm for early diagnosis of vitamin B_{12} in elderly people. Nutr Hosp 2013;28:1447-52.
22. Clarke R, Grimley Evans J, Schneede J, Nexo E, Bates C, Fletcher A, et al. Vitamin B12 and folate deficiency in later life. Age Ageing 2004;33:34-41.
23. Clarke R, Sherliker P, Hin H, Nexo E, Hvas AM, Schneede J, et al. Detection of vitamin B12 deficiency in older people by measuring vitamin B12 or the active fraction of vitamin B12, holotranscobalamin. Clin Chem 2007;53:963-70.
24. Carmel R, Green R, Jacobsen DW, Rasmussen K, Florea M, Azen C. Serum cobalamin, homocysteine, and methylmalonic acid concentrations in a multiethnic elderly population: Ethnic status and sex differences in cobalamin and metabolite abnormalities. Am J Clin Nutr 1999;70:904-10.
25. Wolters M, Hermann S, Hahn A. B vitamin status and concentrations of homocysteine and methylmalonic acid in elderly German women. Am J Clin Nutr 2003;78:765-72.
26. Departemen Kesehatan Republik Indonesia. Angka Kecukupan Gizi bagi bangsa Indonesia. Jakarta: Depkes 2013.
27. Milart P, Wozniakowska E, Wrona W. Selected vitamins and quality of life in menopausal women. Menopause Rev 2018;17(4):175-79. DOI: https://doi.org/10.5114/pm.2018.81742.
28. Shipton MJ, Thachil J. Vitamin B12 deficiency- A 21st century perspective. Clin Med 2015;15(2):145-50.
29. Arendt JFB, Nexo E. Unexpected high plasma cobalamin. Clin Chem Lab Med 2013;51(3):489-96. DOI: 10.1515/cclm-2012-0505.

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