Determining Functional Vitamin B12 Deficiency in the Elderly

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Background: Elevated concentration of serum total homocysteine usually occurs in vitamin B-12 deficiency. This metabolite can be measured and used for screening functional vitamin B-12 deficiency.

Objectives: We assessed functional vitamin B12 deficiency in Tehranian elderly admitted to elderly research center, University of Social Welfare and Rehabilitation Sciences.

Patients and Materials: A cross-sectional study was performed on 232 elderly admitted to elderly research center in Tehran, Iran in 2012. According to other studies, individuals were classified into two groups: high risk of vitamin B-12 deficiency (< 220 pmol/L) and borderline vitamin B-12 (220–258 pmol/L) accompanied by elevated homocysteine (> 15 micmol/L).

Results: Cut-off of 15.0 pmol/L for homocysteine was identified for persons with normal or elevated concentrations. Among persons aged 65–74 and ≥ 75 years, respectively, 56% and 93% were at high risk of vitamin B-12 deficiency.

Conclusions: The prevalence of B12 deficiency was higher in this study compared to other studies, so more attention and massive efficacious policy should be designed to reduce the deficiency of this vitamin.

Keywords: Vitamin B12 Deficiency; Homocysteine; Elderly; Concentration

1. Background

According to the findings of previous studies, malnutrition is the most common cause of vitamin B12 deficiency, but the metabolism of this vitamin may be affected by other factors such as smoking and alcohol consumption, diseases, drugs and enzyme defects etc. (1). Although, this deficiency may be developed in persons with low body storage of vitamin B12 As well as people who have malabsorption or defected metabolism of vitamin B12 and those with physiological conditions with increased demands such as pregnancy and breastfeeding. Long-term deficiency of this vitamin causes different diseases such as neurological and gastrointestinal disorders and anemia (2, 3). Moreover, absorption and reabsorption of vitamin B12 is decreased because of ageing of intestinal mucosa (4, 5). The frequency of high total serum homocysteine is related to age and hyperhomocysteinemia is common in old people and negatively correlated with cognitive status. It may be that deficiency of folate, vitamin B6 and vitamin B12 have an important role in cognitive impairment in the elderly through hyperhomocysteinemia (6). On the other hand, elevated plasma homocysteine concentration is a sensitive marker to evaluate vitamin B12 and folate deficiency. Elevated circulating total homocysteine (tHcy) concentration is a risk factor for cardiovascular disease. In addition, HHcy in the elderly is strongly associated with depression, impaired cognitive function and dementia (7-10). Few studies demonstrated that slight cognitive impairment might be an early symptom. However, few studies were published on other possible early signs and symptoms. In clinical practice, vitamin B12 deficiency is often suspected in elderly patients reporting vague symptoms (1).

2. Objectives

The aim of this study was to determine the frequency of vitamin B12 deficiency in the elderly population of Tehran, using serum total homocysteine as the indicator of tissue vitamin deficiency.

3. Patients and Materials

The study population comprised a random sample of 232 elderly inhabitants of Tehran aged ≥ 65 years who admitted to the elderly research center, University of Social Welfare and Rehabilitation Sciences. This study was performed in Tehran, Iran in 2012. The samples were

collected from two centers by considering a = 0.05 and d = 0.06 in the elderly with about 15% of folic acid and vitamin B12 deficiency, and the factor/coefficient of two was considered for the design effect of sample size formula. This ratio was calculated for about 300 people. However, due to financial limitations because of tariff increases, 232 elderly people were enrolled in the study. Medical history (including pernicious anemia), smoking, consumption of multivitamin supplements and other aspects of health and lifestyle of participants were collected. Venous blood was obtained from all participants into evacuated serum tubes to analyze vitamin and metabolites status and in another evacuated test tube containing EDTA for a full blood count. An insulated box was used to transfer blood samples to the laboratory where the samples were stored at -20°C for 0.5-2 hours before centrifugation. Whole blood samples were centrifuged immediately (3000 grams, 10 minutes, 24°C) after arrival and serum samples were stored at -80°C. An informed written consent was obtained from all participants before entering the study. Serum homocysteine concentrations were measured using a fluorescence polarization immunoassay and an autoanalyzer. Repeated homocysteine measurements were performed in 100 individuals by gas chromatography-mass spectrometry (GCMS) at another laboratory and the correlation coefficient between the GCMS and FPIA homocysteine assays was 0.99. MMA assays were not performed because of technical problems. Vitamin B-12 determination was made by a competitive protein binding assay with an automated chemiluminescence detection system with an analytic imprecision of < 10%. Individuals were classified as high-risk for vitamin B-12 deficiency if they had very low levels of vitamin B-12 (< 150 pmol/L). According to another classification, high-risk was defined as low (< 220 pmol/L) or borderline vitamin B-12 (200–220 pmol/L) concentrations accompanied by elevated homocysteine (> 15 mol/L) concentrations (11).

3.1. Statistical Analysis

Continuous variables were presented as means and SDs. Differences in the mean values of biochemical indexes were compared by analysis of covariance or tests for linear trend across groups. Variables without a normal distribution were transformed to the logarithmic form. Associations of homocysteine with age, gender, vitamin B-12 and creatinine were assessed by spearman correlation. All analyses were performed with SPSS version 15 (IBM,USA).

4. Results

Totally, 232 patients who the inclusion criteria entered the study. Of them, 102 (44%) were male. The mean age of participants was 73.8 ± 5 years [< 75 years: n = 146 (62.9%), >75 years: n = 86 (37.1%)]. Table 1 shows the characteristics of the study population and Table 2 presents the charac-

teristics by age and gender. The mean serum concentration of homocysteine was higher in the older age group than the younger age group, while the mean B12 concentration was higher in the younger age group (P < 0.001). Mean homocysteine and hemoglobin concentrations were higher in men than women (P < 0.001). The correlation between metabolite and age in all participants is shown in Table 3. As this table shows, there was a significant negative correlation between age and hemoglobin and B12, and between B12 and homocysteine; besides, there was a statistically significant positive correlation between age and MCV, and between creatinine and homocysteine (P = 0.001). The frequency (%) of vitamin B12 deficiency in all patients regarding B12 < 150 pmol/L and < 220 pmol/L was 50 (21.6%), 93 (41.1%), respectively. The prevalence (%) of patients with borderline deficiency and homocysteine ≥ 15 micmol/L was 18 (7.8%). Therefore, total prevalence of B12 deficiency, considering this borderline group with an elevated homocysteine level (≥ 15 micmol/L) and those with B12 < 220 pmol/L, was 111 (47.9%), known as high-risk subjects. The frequency (%) of vitamin B-12 concentrations for each age and gender-specific category is separately shown in Table 4. In men, the prevalence of normal (≥ 258 pmol/L), borderline (220-258 pmol/L), low (< 220 pmol/L) and very low (< 150 pmol/L) vitamin B-12 concentrations were 49%, 12.7%, 38.2% and 20.6%, respectively. The prevalence of normal to very low concentrations of vitamin B were 30.2%, 15.1%, 54.7% and 31.4% in older (≥ 75) and 60.3%, 8.2%, 31.5% and 15.8% in younger patients (< 75), respectively. There was a downward shift to lower B12 concentrations in older patients. Thus, 56% of patients aged 65-74 years and 93% of those aged ≥ 75 years were at high-risk of vitamin B-12 deficiency. As mentioned earlier, there was a negative correlation between homocysteine and concentrations of vitamin B-12 (r = -0.396). As shown in Table 5, the prevalence of very low (< 150 pmol/L), low (< 220 pmol/L) and borderline (220 - 258 pmol/L) B12 levels were 10, 5 and 3 times higher in the category with elevated homocysteine (≥ 15 micmol/L), respectively.

Table 1. Characteristics of the Study Population[a]

Variables	
Age, y	73.7 ± 5.2
B12, pmol/L	295 ± 170
Homocysteine, micmol/L	19 ± 7
Hemoglobin, g/dL	14 ± 1.7
MCV, FL	92 ± 7.2
Creatinine, mg/dL	1 ± 0.2

[a] Data are presented as Mean ± SD.

Table 2. Characteristics of the Study Population by Age and Gender

Variable	Male (n = 102)		Female (n = 130)		P Value	
	< 75	≥ 75	< 75	≥ 75	Age	Gender
Age, y	69 ± 3	79 ± 3	71 ± 2	79 ± 2		
B12, pmol/L	320 ± 157	2480 ± 150	328 ± 140	242 ± 145	0.001	0.46
Homocysteine, micmol/L	20.6 ± 7	20 ± 7	17 ± 7	19.5 ± 6	0.89	0.002
Hemoglobin, g/dL	15 ± 1	14 ± 1	13 ± 1	13 ± 1	0.001	0.002
MCV, FL	91 ± 7	91.1 ± 7	92 ± 6	93 ± 6	0.23	0.94
Creatinine, mg/dL	1.7 ± 0.3	1.7 ± 0.3	1.4 ± 0.4	1.5 ± 0.5	0.32	0.19

Table 3. Possible Correlation Between Different Variables of the Study Population

Variable	Correlation Coefficient[a]	P-Value[b]
Age		
Hemoglobin	-0.339	0.001
MCV	+0.161	0.001
B12	-0.337	0.001
Creatinine		
Homocysteine	+0.285	0.001
B12		
Folate level	+0.269	0.001
Homocysteine	-0.396	0.001
Folate Level		
Homocysteine	-0.24	0.001

[a] Spearman correlation.
[b] P-value is significant less than 0.05.

Table 4. Total Prevalence of B12 Deficiency and Its Prevalence by Age and Gender

Cutoff Variable	Age		Gender		All Patients
	< 75	≥ 75	Male	Female	
B12, pmol/L					
< 150	23 (15.8)	27 (31.4)	21 (20.6)	29 (22.3)	50 (21.6)
< 220	46 (31.5)	47 (54.7)	39 (38.2)	54 (41.5)	93 (40.1)
220 - 258	12 (8.2)	13 (15.1)	13 (12.7)	12 (9.2)	25 (10.8)
≥ 258	88 (60.3)	26 (30.2)	50 (49)	64 (49.2)	114 (49)

Table 5. Prevalence of B12 Deficiency Regarding Homocysteine Level

Homocysteine, micmol/L	B12 Level, pmol/L			
	< 150	< 220	220 - 258	≥ 258
< 15	5 (2.2)	16 (6.9)	7 (3)	50 (21.6)
≥ 15	45 (19.5)	77 (33.2)	18 (7.8)	64 (27.6)

5. Discussion

Vitamin B-12 deficiency is common in the elderly population. Typical causes of cobalamin deficiency in the elderly include pernicious anemia and food-bound malabsorption, but these causes explain less than a half of low vitamin B-12 concentrations. As shown in this study, there was a negative correlation between B12 concentration and patients' age. The results of the present study showed that the prevalence of vitamin B-12 deficiency was similar in both genders, which is in contrast with previous reports (12, 13). Concentrations of homocysteine were elevated among those who had the lowest vitamin B-12 concentrations. Therefore, use of homocysteine in individuals with borderline vitamin concentrations may identify those with functional deficiency and those for whom treatment is indicated.

Table 6. Prevalence of B12 Deficiency Regarding Homocysteine Level

Homocysteine	B12 Level
< 15	
	220
	220 - 258
	> 258
≥ 15	
	220
	220 - 258
	> 258

Measurement of serum or plasma cobalamin concentration is more specific, but varies depending on the method and particular laboratory. In adults, a concentration of 150 pmol/L (200 pg/mL) is considered the lowest level for an adequate supply. In a developing deficiency, serum concentrations are maintained by depleting the stores of vitamin. Therefore, concentrations above the cut-off value of 150 pmol/L do not inevitably reflect a sufficient vitamin B12 status as shown in some studies, indicating that concentration is not a significant variable (14). On the other hand, if the cobalamin concentration is below this cut-off value, depleted stores can be assumed but not necessarily present (15). Based on studies, a serum cobalamin cutoff value of < 220 pmol/L (< 300 pg/mL) has been suggested, with more sensitive indicators of cobalamin status such as methylmalonic acid (MMA) and homocysteine (see below). Lindenbaum et al. (16) suggested a cut-off value of 220-258 pmol/L (300-350 pg/mL) in the elderly population (16, 17). Individuals with low vitamin B12 concentrations exhibited significant elevations of MMA and homocysteine concentrations. However, some authors believe that the cut-off value of 150 pmol/L is too low. For example, in a sample of elderly patients with cobalamin concentrations below this value, 40% of subjects had increased serum MMA levels. In another study, as many as 80% of subjects aged 65 years with cobalamin concentrations > 148 pmol/L had increased MMA and homocysteine values (15, 16). Vazquez-Pedrazuela et al. observed vitamin B12 deficiency in 16.5% of the study participants (a population of 65 years and over). A strong association was found between vitamin B12 deficiency and cardiac and cerebrovascular diseases, vascular risk factors and drugs administered in prevention of cardiac events and ischemic stroke. This study showed a higher prevalence of vitamin B12 deficiency in the elderly population compared to the literature (18). In our study, a threshold of 220 pmol/L (300 pg/mL) was recognized as a desirable status indicator in the elderly (15, 18, 19). In case of concentrations below 220 pmol/L, further diagnostic measures are necessary. These early indicators of cobalamin deficiency could be elevated plasma homocysteine, elevated serum MMA and decreased serum holotrans-

cobalamin (holoTC) (15, 20, 21). Elevated homocysteine is an important indicator of vitamin B12 deficiency and may indicate a low vitamin B6 status. In elderly with normal folate and vitamin B6 levels, elevated homocysteine is generally a consequence of cobalamin deficiency (15, 22). Our study showed an inverse association that was approximately linear and graded in the proportions with elevated homocysteine with decreasing concentrations of vitamin B-12. However, the cuto-ff point of > 15 micmol/L for homocysteine can be used to distinguish individuals with elevated concentrations from those with normal concentrations. Different levels of total homocysteine in plasma were suggested as normal: <14 micmol/L (23, 24) 5-13.6 micmol/L (25, 26) and 4.9 – 11.7 micmol/ L (27, 28). Since many studies showed that the prevalence and mortality of cardiovascular disease (CVD) increased if a concentration of 10 micmol/L was exceeded (28-31), this limit has been suggested as desirable (32, 33). Values of > 12 - 30 micmol/L are classified as moderate hyperhomocysteinemia (22, 34). Although MMA is a more specific and sensitive indicator of cobalamin status, we did not have the required kits for measuring its level in our patients. Elevated MMA is a direct metabolic consequence of vitamin B12 deficiency (35, 36). Therefore, MMA is an important biochemical marker of cobalamin status (16, 19, 25, 26, 37, 38). The reference range of serum MMA concentrations in healthy adults is 73-271 nmol/L (39, 40). In elderly individuals, the prevalence of subnormal cobalamin concentration varies between 10% and 43% depending on the diagnostic criteria (31, 39, 41, 42). If the previously considered threshold of 150 pmol/L (200 pg/mL) for a normal vitamin B12 status is used, only 10-15% of the elderly population is classified as cobalamin deficient, which is about 21.6% of the cobalamin deficient patients in this study. This shows significant difference of B12 deficiency with other countries, even with this cut-off point (< 150 pmol/L). A study showed that the prevalence of combined B12 insufficiency and supraphysiological concentrations of serum folate may have increased with folic acid food fortification in Canadian women aged 65 years and older who underwent concomitant clinical testing of serum folate and B12 (43). The threshold of < 220 pmol/L (< 300 pg/mL) is considered as a desirable status indicator in the elderly (15, 16, 32, 34, 35) and otherwise more sensitive markers such as blood concentration of homocysteine or methylmalonic acid are used. Then, the prevalence of cobalamin deficiency rises to up to 43% (4, 44-46). According to our study, a possible approach to screen vitamin B-12 deficiency starts with measurement of vitamin B-12. If individuals have vitamin B-12 concentrations below 220 pmol/L, more detailed investigations should be undertaken to discover the underlying cause and the best treatment. If patients have vitamin B-12 concentrations between 220 and 258 pmol/L, homocysteine may assist in identifying those who require more detailed examination and treatment. Therefore, in this case, the frequency (%) of patients with cobalamin deficiency would be about

47.9%, which is higher than other studies (43%) (14, 15, 19, 32). According to the findings of previous studies, use of supplements or enriched foods reduces the prevalence of cobalamin deficiency in the elderly (15, 41, 42, 47). In Rajan et al. study on elderly aged 65-100 years, 46% of screened patients reported regularly taking a source of synthetic cobalamin. Nevertheless, 13% were classified as vitamin B12 deficient because of serum cobalamin concentrations < 220 pmol/L and MMA concentrations > 271 nmol/L. In the elderly, supplementation with less than 50 micg/day vitamin B12 does not seem to prevent a poor cobalamin status (19). Because of the high prevalence of vitamin B12 deficiency and its association with neurocognitive diseases, daily use of cobalamin supplements of > 50 micg/day is recommended for elderly people aged 60 years and older. This preventive measure has no side effects and would be useful with respect to a risk-benefit analysis. Due to insecure cobalamin supply, it is necessary to monitor the status of vitamin B12 regularly in elderly people (> 60 years). Since serum vitamin B12 is not a reliable indicator of subclinical cobalamin deficiency, measurement of more sensitive indicators like MMA and homocysteine should be considered. In patients with diagnosed cobalamin deficiency, the above mentioned preventive doses of cobalamin are insufficient (15). Shobha et al. in their study highlighted the association of dietary habits of urban south Indian elderly and plasma vitamin B12, folate and homocysteine levels with cognitive status and impact of vitamin supplementation on normalization of the same. They found that daily vitamin B12 intake was 3.5 times higher than the FDA recommendation contrary to the belief that dietary intake would be low in Indian population as many are vegetarian (57%). However, no correlation was detected between plasma vitamin B12 and cognitive status in elderly population (48). Conclusions: The prevalence of vitamin B12 deficiency was higher in this study compared to other studies. Considering the consequences of vitamin B12 deficiency, more attention should be paid to this vitamin and preventive measures should be devised to reduce its deficiency.

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Authors' Contributions

Nilufar Khodabandehloo: performed the experimental work; Masoud Vakili and Zahra Hashemian: planning of the project and analysis of data; Hadi Zare Zardini: writing the paper.

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