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The relationship between vitamin B12, folate and homocysteine levels in the elderly Turkish population

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Abstract

Aging is associated with increased incidence and prevalence of chronic diseases, and the role of micronutrient deficiencies in the development of diseases is important. Vitamin B12 and folic acid deficiency are often associated with increased homocysteine (Hcy) levels. In our study, we aimed to detect the relationship between Hcy levels by examining vitamin B12 and folate levels in the healthy elderly Turkish population. In our study, the levels of vitamin B12, folate, and Hcy were analyzed and examined in 657 elderly and 642 non-elderly healthy adults admitted to the Internal medicine outpatient clinic. Vitamin B12 <200 pg/mL was considered to be deficient. Folic acid <5 ng/mL was considered a deficiency, and Hcy>15 μmol/L was considered a high concentration. Vitamin B12 levels were detected to be significantly decreased in the elderly group compared to the non-elderly group, while Hcy levels were marked to be increased ($p<0.05$). Women had lower levels of Hcy and higher levels of vitamin B12 and folate ($p<0.05$). There was a moderate negative correlation between Hcy and vitamin B12 levels in the elderly group ($r=-0.576$; $p<0.0001$), and a moderate negative correlation between Hcy and folate ($r=-0.510$; $p<0.0001$). In the elderly group, 21.5% had vitamin B12 deficiency and 21.6% had folate deficiency. An increase in Hcy level was detected in 54.8%. Those with Hcy levels >15 μmol/L had vitamin B12 deficiency in 38.4%, folate deficiency in 36.0%, and both vitamin B12 and folate deficiency in 15.8%. Our results indicate that it is important to measure vitamin B12, folate, and Hcy in the elderly, given the significant growth in the elderly population. We predict that vitamin B12 and folate supplementation, when necessary, may be beneficial in preventing some common diseases in the elderly and increasing the standard of life of the elderly and their relatives.

Keywords: Homocysteine, vitamin B12, folic acid, elderly

Introduction

Aging is a process characterized by the development of many pathophysiologies, including cardiovascular and neuropsychiatric diseases, as a result of the loss of physiological integrity [1]. Vitamin deficiencies are observed more frequently due to reasons such as nutritional problems, chronic diseases, and high frequency of drug use in the elderly population [2]. Due to the difficulty in estimating dietary intake in the elderly and age-related changes that may affect the bioavailability of nutrients, nutritional status should not be evaluated only with dietary information, but also clinically and biochemically [3].

Among the vitamin deficiencies seen in the elderly, especially vitamin B12 and folic acid deficiencies are common [4]. The prevalence of vitamin B12 deficiency varies between 10% and 43% in the elderly, as the cut-off values are different [5]. However, since the clinical manifestations of vitamin B12 deficiency are highly variable, it is usually difficult to evaluate [2]. Vitamin B12 deficiency can cause hematological disorders, neural tube defects, cardiovascular diseases, and nervous system pathologies [6]. In addition, symptoms that are not specific to old age, such as dizziness, weakness, fatigue, and impaired cognitive function, may also occur [7]. The clinical severity of vitamin

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B12 deficiency is generally not related to vitamin B-12 levels [8]. In some studies, vitamin B12 deficiency has been found to be associated with folate deficiency [5]. It is stated that in addition to vitamin B12 deficiency in the elderly, 40% folate deficiency is observed, and homocysteine (Hcy) levels increase in 84% of people with folate levels below normal [4]. Folate deficiency causes some disorders such as macrocytic anemia, cardiovascular diseases, depression, and Alzheimer's; however, it differs from vitamin B12 deficiency with different neurological findings [6].

Homocysteine, a thiol-containing amino acid, occurs during the metabolism of methionine [2]. Hyperhomocysteinemia has been shown to be associated with atherosclerotic and thrombotic vascular disease independently of other risk factors [9]. Vitamin B12, along with folate, plays a role as a cofactor in the conversion of homocysteine to methionine. Therefore, folate and vitamin B12 deficiency lead to an increase in Hcy levels [7]. It has been stated that high Hcy concentrations are an important marker of vitamin B12 and folate deficiency and that vitamin B12 and folate replacement markedly reduce Hcy levels [10].

Vitamin B12 and folic acid deficiencies are more common in the elderly due to age-related physiological changes, malnutrition, and a high incidence of atrophic gastritis. Early detection and treatment of this condition is extremely important as it will prevent irreversible damage. The purpose of our study is to examine serum vitamin B12, folate, and Hcy levels and the relationship between them in healthy elderly individuals.

Material and Methods

This study was conducted with 1299 healthy Turkish adults who applied to the Internal Medicine outpatient clinic of Başakşehir Çam and Sakura City Hospital between April 2022 and 2023. Individuals taking regular vitamins, pregnant women, cardiovascular disease, cerebrovascular disease, renal failure, cancer, and chronic intestinal diseases were excluded from the study. The cases were divided into two groups according to their age <60 years (642) and ≥60 years (657). Vitamin B12 <200 pg/mL was considered a deficiency. Folic acid <5 ng/mL was considered a deficiency, and Hcy >15 µmol/L was considered a high concentration.

This study complies with the Declaration of Helsinki. This study was approved by the Istanbul Başakşehir Çam and Sakura City Hospital ethics committee (No:2023.06.264). Due to the retrospective and observational character of the study design, the requirement for informed consent has been waived.

Data collection and analysis

All data belonging to the patients were obtained from the hospital information system and compared. All tests were analyzed in the central laboratory of Istanbul Başakşehir Çam and Sakura City Hospital.

Serum glucose level was measured with enzymatic reference with hexokinase, creatinine level was measured with kinetic colorimetric method in Roche Diagnostics Cobas 8000 (Roche Indianapolis/America) analyzer. Interassay and intraassay CV values were 0.75% vs 0.63% for glucose; 1% vs 0.87% for urea; and 1.7% vs 1.46% for creatinine. Homocysteine levels were measured with the homocysteine enzymatic assay method, vitamin B12, and folic acid concentrations were measured with the competition principle on the Roche Diagnostics Cobas 8000 (Roche Indianapolis/America) analyzer. Interassay and intraassay CV values were 1.2%, 1.6% for Hcy; 1.95%, 3.6% for vitamin B12; and 4%, 4.5% for folate.

The data were examined by the SPSS Version 26.0 statistical package program (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.). Categorical variables were presented as numbers and percentages, continuous variables were presented as "mean±SD" and "median (minimum–maximum)" values. The compatibility of continuous variables with normal distribution was assessed with the Kolmogorov-Smirnov test, and the Mann-Whitney U test was used for comparisons between groups. The comparison of categorical variables between groups was made with the Chi-Square test. The relationship between Homocysteine, vitamin B12, and folic acid levels was assessed with the Spearman Rho correlation test. The statistical significance level was accepted as "$p<0.05$" in all analyses.

Results

Of the 1299 patients included in the study, 657 were elderly (≥60 years) and 642 were in the non-elderly group (<60 years). Of the elderly group, 42.9% (282) were male, 57.1% (375) were female; 41.9% (269) of the non-elderly group were male and 58.1% (373) were female. There was no difference among the two groups in terms of gender distribution ($p>0.05$). When the laboratory parameters were evaluated, no statistically significant difference was found between the folate levels of the two groups ($p>0.05$). While homocysteine (16.3±7.0 µmol/L, 11.5±3.2 µmol/L), glucose (91.8±9.9 mg/dL, 87.2±7.3 mg/dL), and creatinine (0.82±0.17 mg/dL, 0.72±0.14 mg/dL) levels were higher in the elderly group, vitamin B12 levels were lower in the elderly group (322.8±121.6 pg/mL, 386.1±190.2 pg/mL) ($p<0.0001$) (Table 1). The distribution of Hcy, vitamin B12, and folate levels in the elderly and non-elderly groups is shown in Figure 1.

Table 2; shows the distribution of Hcy, vitamin B12, and folic acid levels in the elderly group by gender. Hcy level is lower in women than men (14.9±5.9 µmol/L, 18.0±7.8 µmol/L), vitamin B12 and folic acid levels are higher in women than men (342.1±128.4 pg/mL, 297.1±106.8 pg/mL; 8.02±4.72 ng/mL, 6.23±3.14 ng/mL respectively) ($p<0.0001$).

The correlation of all three parameters with age is shown in Figure 2. There was a positive weak correlation between age and Hcy level ($r=0.230$; $p<0.0001$); a very weak negative correlation between age with vitamin B12 and folic acid levels ($r=-0.087$; $p=0.026$ and $r=-0.130$; $p=0.001$ respectively) (Figure 2).

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When the relationship among Hcy, vitamin B12, and folic acid levels in the elderly group was evaluated, there was a moderate negative correlation between Hcy and vitamin B12 levels (r=-0.576; p<0.0001); a moderate negative correlation between hcy and folic acid levels (r=-0.510; p<0.0001); a weak positive correlation (r=0.330; p<0.0001) was found between vitamin B12 and folic acid (Figure 3).

Table 1. Laboratory data of the elderly and non-elderly group

	Group		p value
	Elderly	**Non-elderly**	
Gender	**n (%)[a]**	**n (%)[a]**	
Male	282 (42.9)	269 (41.9)	0.709[b]
Female	375(57.1)	373 (58.1)	
	n Mean±SD Median (Min-Max)	**n Mean±SD Median (Min-Max)**	
Homocysteine (µmol/L)	657	642	<0.0001[c]
	16.3±7.0	11.5±3.2	
	14.8 (5.9-80.8)	10.9 (3.7-19.9)	
Vitamin B12 (pg/mL)	657	630	<0.0001[c]
	322.8±121.6	386.1±190.2	
	307 (100-695)	338.5 (126-1674)	
Folic acid (ng/mL)	657	622	0.090[c]
	7.26±4.21	8.02±5.72	
	6.22 (0.89-35.20)	6.57 (1.89-40.00)	
Glucose (mg/dL)	569	469	<0.0001[c]
	91.8±9.9	87.2±7.3	
	93.0 (65-110)	88 (68-108)	
Creatinine (mg/dL)	637	623	<0.0001[c]
	0.82±0.17	0.72±0.14	
	0.82 (0.44-1.16)	0.71 (0.39-1.11)	

[a]Column percentage, [b]Chi-square test, [c]Mann-Whitney U test

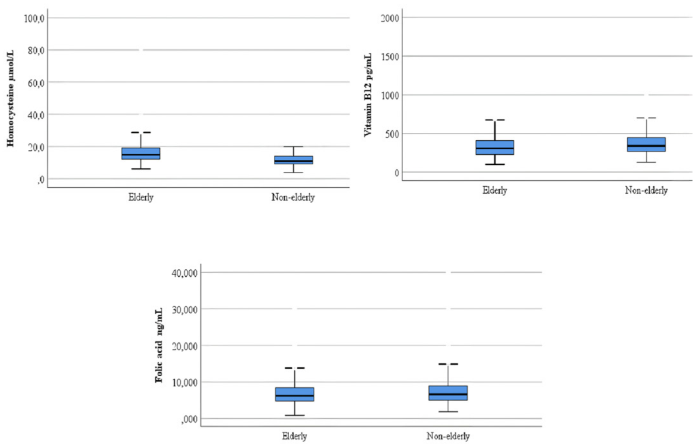

Figure 1. Homocysteine, vitamin B12 and folic acid levels of elderly and non-elderly groups

Table 2. Homocysteine, vitamin B12 and folic acid levels of elderly group according to gender

	Female (n=375)	Male (n=282)	
	n Mean±SD Median (Min-Max)	**n Mean±SD Median (Min-Max)**	**p value**
Homocysteine (µmol/L)	657	642	<0.0001[a]
	14.9±5.9	18.0±7.8	
	13.9 (5.9-80.8)	16.4 (7.0-75.2)	
Vitamin B12 (pg/mL)	657	630	<0.0001[a]
	342.1±128.4	297.1±106.8	
	327.0 (100-695)	288.5 (100-634)	
Folic acid (ng/mL)	657	622	<0.0001[a]
	8.02±4.72	6.23±3.14	
	6.99 (0.90-35.20)	5.55 (1.35-28.40)	

[a]Mann-Whitney U test

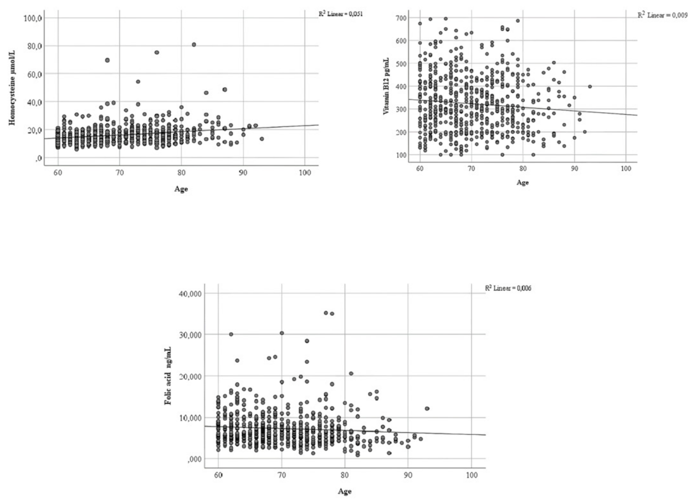

Figure 2. Age and homocysteine, vitamin B12 and folic acid correlations among the elderly groups

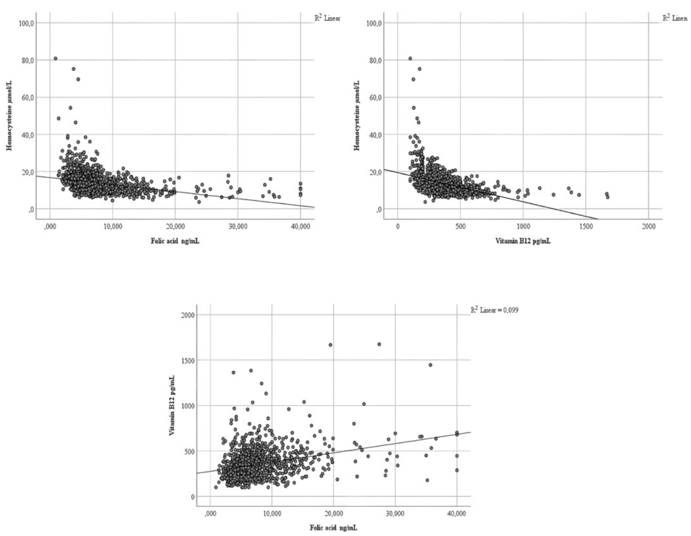

Figure 3. Homocysteine, vitamin B12, and folic acid correlations

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Table 3 shows the levels of Hcy, vitamin B12, and folic acid in the elderly and non-elderly groups. The percentage of those with Hcy levels >15 µmol/L is higher in the elderly group (45.2%; 13.6%) ($p<0.05$) and the percentage of those with a vitamin B12 level <200 pg/mL is higher in the elderly group than in the non-elderly group (21.5%; 8.6%) ($p<0.05$). There was no statistically significant difference between the groups in terms of the percentage of patients with folic acid <5 ng/mL ($p>0.05$).

Table 3. Homocysteine, vitamin B12 and folic acid levels in elderly and non-elderly groups

	Group		p value[b]
	Elderly	Non- elderly	
	n (%)[a]	n (%)[a]	
Homocysteine			
>15 µmol/L	297 (45.2)	87 (13.6)	<0.0001
≤15 µmol/L	360 (54.8)	555 (86.4)	
Vitamin B12 (pg/mL)			
<200 pg/mL	141 (21.5)	54 (8.6)	<0.0001
≥ 200 pg/mL	516 (78.5)	576 (91.4)	
Folic acid			
<5 ng/mL	142 (21.6)	128 (20.6)	0.650
≥5 ng/mL	515 (78.4)	494 (79.4)	

[a]column percentage, [b]Chi-square test

The distribution of vitamin B12 and folic acid concentrations in the elderly group with Hcy levels >15 µmol/L and ≤15 µmol/L is shown in Table 4. The levels of both parameters are lower in the elderly with Hcy level >15 µmol/L ($p<0.05$) (Table 4 and Figure 4).

Table 4. Vitamin B12 and folic acid levels in the elderly with homocysteine >15 µmol/L and ≤15 µmol/L

	Homocysteine		
	>15 µmol/L (n=297)	≤15 µmol/L (n=360)	
	n Mean±SD Median (Min-Max)	n Mean±SD Median (Min-Max)	
Vitamin B12 (pg/mL)	657 258.7±85.8 253.0 (100-490)	630 375.7±121.4 376.0 (100-695)	<0.0001
Folic acid (ng/mL)	657 5.83±3.39 5.28 (0.89-35.20)	622 8.44±4.46 7.34 (2.13-35.0)	<0.0001

[a]Mann-Whitney U test

Of the 297 elderly people with Hcy levels >15 µmol/L, 114 (38.4%) had a vitamin B12 level of less than 200 pg/mL, and 107 (36.0%) had a folic acid level of less than 5 ng/mL. 47 (15.8%) of the elderly with Hcy concentrations higher than 15 µmol/L also have low vitamin B12 and folic acid levels.

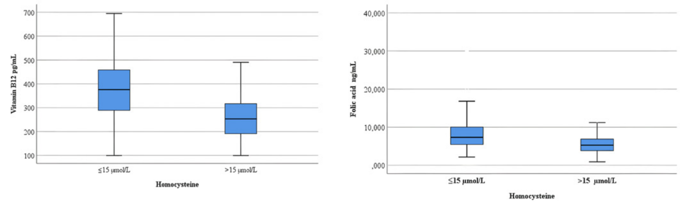

Figure 4. Vitamin B12 and folic acid levels of the elderly with homocysteine levels >15 µmol/L and ≤15 µmol/L

Discussion

Living conditions, developments in science and technology have contributed to the increase in the elderly population [6]. Aging is a condition characterized by the gradual loss of physiological integrity and the development of many pathophysiologies, including cardiovascular and neuropsychiatric diseases [1]. Vitamin deficiencies are observed more frequently in the elderly population [2]. It is stated that especially vitamin B12 and folate deficiencies are common in elderly individuals and increase with age [7]. In our study, we found that vitamin B12 levels were markedly decreased in the elderly group compared to the non-elderly group, while Hcy levels were increased. We found a negative correlation between Hcy and vitamin B12 and folate in the elderly group. There was a positive correlation between age and Hcy level; a negative correlation between age with vitamin B12 and folate levels.

Vitamin B12 deficiency has been reported to be common in underdeveloped countries and in the elderly [11]. More than 90% of patients do not show signs of vitamin B12 deficiency, which causes progressive neurological and hematological diseases [12]. The prevalence of vitamin B12 deficiency in the elderly has been found to be 5-25% [1]. The reason for this variability is the use of different cutoff values [13]. In a study by Miller et al. The cutoff value for vitamin B12 deficiency was <148 pmol/L, Fakhrzadeh et al. reported it as <190 pmol/L [14-15]. In a study examining the vitamin B12 level in the elderly, vitamin B12 deficiency was found to be 62.2% between the ages of 60-74; vitamin B12 72.2% between 75-84 years old and 50% at 85 years and older [6]. In our study, we found that vitamin B12 levels were statistically markedly decreased in the elderly compared to the non-elderly. Although the causes of vitamin B12 deficiency in the elderly have not yet been fully elucidated, dietary habits, a decrease in dietary vitamin B12 intake, and 30-40% cobalamin malabsorption are shown as possible causes [2]. Hereditary cobalamin metabolism diseases and some drugs (proton pump inhibitors, some antiepileptic drugs, antibiotics, and antidiabetic drugs) also cause vitamin B12 deficiency [16].

In studies, vitamin B12 levels were detected to be lower in the elderly with health problems or living in a nursing home than in the socially active elderly [17]. In one study, vitamin B12 levels were found to be low in 32% of geriatric patients [6]. In our study, when vitamin B12 <200 pg/mL was considered a deficiency, we found that 21.5% of the elderly group had vitamin B12 deficiency. However, Fenech et al. stated that when serum vitamin B12 levels are below 300 pmol/L, the B12 level should be above 300 pmol/L because DNA damage occurs [18]. Ray et al. found that vitamin B12 levels were higher in women than men [19]. In our study, we found that vitamin B12 levels were lower in the male elderly group than in females, consistent with previous reports [7].

Due to the joint participation of vitamin B12 and folate in the methionine synthase reaction, folate concentrations are also affected in vitamin B12 deficiency, and folate deficiency develops in 40% of individuals with vitamin B12 deficiency [1]. While folate deficiency is rare in adults, subclinical folate deficiency is more common in the elderly [20]. In our study, although serum folate was lower in the elderly group compared to the control group, it was not statistically significant. It has been reported that the prevalence of folate deficiency increases with age in individuals aged 65 and over, affecting 5-20% of elderly individuals in the UK [21]. According to Yıldırım et al., 10.4% between the ages of 60-74; Folate deficiency was found to be 12.2% between the ages of 75 and 84 years and 20% at the age of 85 and above [22]. Similar to the literature, we also found folate deficiency in 21.6% of the elderly group [2,4]. We also detected that folate levels were lower in males than females in our study, confirming previous study findings [19]. This is significant because of the association of folate deficiency with depression and dementia in the elderly [11]. Herrmann et al. have seen a positive correlation between vitamin B12 and folate levels, similar to our results [23]. It has been emphasized that the elderly with economic problems may be at greater risk for folate deficiency due to their lower purchasing power [24]. Folic acid deficiency in the elderly develops due to inadequate dietary intake or malabsorption [6]. H. pylori infection causes chronic atrophic gastritis, inflammatory bowel disease, alcoholism, various drugs (trimethoprim, aminopterin, methotrexate, pyrimethamine, and some antiepileptics) may cause folate deficiency [16].

In the elderly, increased Hcy levels are common in the development of cardiovascular and cerebrovascular diseases [1]. Vitamin deficiencies (vitamin B6, vitamin B12, and folic acid) are among the various causes of high Hcy [23]. Therefore, increased Hcy concentrations can be considered an indicator of vitamin B12 and folic acid deficiencies. Bottiglieri et al. in their work; found that Hcy concentrations are increased in depressed middle-aged and elderly individuals [25]. Ray et al. Hcy levels were detected to be higher in males than females [19]. Our results showed that Hcy levels, which we found higher in the elderly, were statistically significantly higher in men than in women. It has been reported that Hcy levels are generally associated with renal functions, age-related decreases or abnormalities in other enzymes involved in Hcy metabolism, and male gender [26]. We found that approximately half of the elderly group had Hcy levels higher than 15 µmol/L. Whereas, the same rate in the non-elderly group was 13.6%, similar to epidemiological studies [27]. Ozdem et al. Similar to the results of their study in 226 elderly and 260 non-elderly adults, we found a positive correlation between Hcy level and age [2].

Vitamin B12 and folate deficiency is characterized by megaloblastic anemia and causes increased SAM and Hcy levels by decreasing methionine synthase activity [20]. Elevated serum Hcy has been found in >90% of subjects with megaloblastic anemia due to vitamin B12 deficiency or folate deficiency [28]. In a study, a decrease in vitamin B12 and folate levels and an increase in Hcy levels were found with aging [19]. In a study involving 164 Alzheimer's patients, Alzheimer's disease was found to be associated with high Hcy and low serum folate and vitamin B12 [29]. Joosten et al. found an increase in Hcy or methylmalonic (MMA) in 75% of people with low vitamin B12 levels [30]. It has been stated that the use of Hcy or MMA will be beneficial in the identification and early treatment of individuals with low vitamin B12 levels, and the concentrations of these parameters will decrease as a result of treatment [7].

Clarke et al. detected a negative correlation between low vitamin B12 levels and high Hcy [7]. In our study, similar to other studies, we found a negative correlation between Hcy and folic acid and vitamin B12 levels in the elderly [2,4]. Both folic acid and vitamin B12 levels were low in 47 (15.8%) of the elderly with hcy levels higher than 15 µmol/L. Therefore, it can be thought that the elderly with vitamin B12 and folate deficiency are at risk for hyperhomocysteinemia. The high rate of hyperhomocysteinemia accompanying vitamin B12 and folate deficiency in the elderly causes a higher incidence of diseases such as cardiovascular diseases and stroke with hyperhomocysteinemia in the etiopathogenesis [16].

Huerta et al. found that the Hcy-lowering effect of folate was seen at serum levels of at least 11 nmol/L [31]. It is stated that folate, which is used as a routine additive in foods such as flour, pasta, and rice in some countries, is effective in reducing Hcy levels [10]. However, high-dose folate supplementation may cause irreversible neurological damage by masking vitamin B12 deficiency [32]. It has been stated that the use of combined vitamin B12 and folic acid in patients with ischemic stroke lowers Hcy levels more than using only one of them, and that combined therapy may be effective in the secondary protection of these patients [33].

The random selection and large sample size of our study are its strengths. However, our study had some limitations. Our study does not reflect the entire Turkish population, as it consists of data from a single center. Food intake assessment of patients is not available. Our retrospective study sheds light on the relationship of vitamin B12, folic acid, and Hcy levels with age. However, this relationship should be supported by multicenter studies with broad participation, including nutritional details.

Conclusion

We found that vitamin B12 and folate concentrations decreased and homocysteine levels increased in the elderly and especially in men. There was a relationship between the increase in homocysteine level and vitamin B12 and folate. Current results

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reveal the importance of routine screening of vitamin B12, folate, and Hcy levels in the elderly. Early detection of changes in these parameters and providing vitamin support when necessary will be beneficial for the prevention, early detection, and treatment of various common diseases seen in elderly individuals. In addition, considering the increase in the elderly population, public health strategies should be developed to increase the awareness of the elderly on balanced nutrition and health. Thus, we believe that this step, which does not require much cost, will increase the standard of life of the elderly and their relatives.

Conflict of Interests

The authors declare that there is no conflict of interest in the study.

Financial Disclosure

The authors declare that they have received no financial support for the study.

Ethical Approval

This study was approved by the ethics committee of İstanbul Başakşehir Çam and Sakura City Hospital (No:2023.06.264).

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