Original Article

Are vitamin B-12 measurements adequate for evaluating its deficiency in individuals?

Sedat Özdemir MD[1] and Selda Demirtaş MD[2]

[1]*Ankara Atatürk Sanatorium Training and Research Hospital, Ankara, Turkey*
[2]*Ufuk University, Dr. Rıdvan EGE Training and Research Hospital, Mevlana Bulvarı (Konya Yolu), Ankara, Turkey*

Background and Objectives: Measurement of vitamin B-12 deficiency using different methods may cause diagnostic difficulties. In order to rapidly and safely diagnose vitamin B-12 deficiency, it is important to determine the reference ranges of serum B-12 and its related biomarkers such as homocysteine, holotranscobalamine (holo-TC) and methylmalonic acid (MMA). This study aimed to determine reference interval (RI)s for serum vitamin B-12 and related markers. **Methods and Study Design:** Samples were collected from 404 young-to-middle-aged healthy adults aged 18-65 years. Vitamin B-12, homocysteine, holotranscobalamin, folate were analyzed using the Arcitect i2000 device. Plasma MMA was analyzed by LC/MS. RIs were then evaluated accordingly. **Results:** Vitamin B-12, folate, homocysteine, holotranscobalamin and plasma MMA were 139-619 pg/mL, 3.0-14.7 ng/mL, 5.6-18.4 μmol/L, 10.7-101 pmol/L, and 0.01-0.8 μmol/L, respectively. Age group-specific RIs were also generated. **Conclusions:** This study revealed that the diagnosis of vitamin B-12 deficiency should not only be based on serum vitamin B-12 levels, but also of folate, homocysteine, holotranscobalamin and MMA levels; all which are related to vitamin B-12 metabolism.

Key Words: vitamin B-12, folate, holotranscobalamin II, methylmalonic acid, homocysteine

INTRODUCTION

Vitamin B-12 (cobalamin) is an essential nutrient required for normal cell activity in the human body. It acts as a coenzyme for hematopoiesis and neuropsychiatric processes, and plays an important role in synthesizing key molecules, including hormones, neurotransmitters, and DNA (Figure 1). Vitamin B-12 is also essential for the nervous system, particularly in nerve metabolism, myelin synthesis, and neuronal regeneration.[1]

Vitamin B-12 deficiency often leads to hematologic disorders, nervous system symptoms, and cognitive dysfunction.[2] Clinically symptomatic cobalamin deficiency is widely regarded as a condition primarily affecting older individuals, with symptoms observed in 1–2% of this population.[3] Vitamin B-12 deficiency in young and middle-aged individuals is diagnosed based on subnormal levels of biochemical biomarkers, often without clinical symptoms. However, studies suggest that such deficiency may lead to declines in cognitive and neurological functions.[4,5] This condition is often overlooked or mistaken for other disorders and is typically confirmed only after detecting a subnormal vitamin B-12 level. This condition, known as subclinical cobalamin deficiency (SCCD), accounts for 10–20% of vitamin B-12 deficiency cases. Diagnosis of SCCD based on clinical findings is challenging and relies solely on biochemical biomarkers.3 A definitive diagnosis of vitamin B-12 deficiency requires multiple biomarkers. While circulating vitamin B-12 levels are commonly measured, variability in these measurements can lead to diagnostic errors. As a result, some experts recommend using functional biomarkers, such as homocysteine or preferably methylmalonic acid (MMA), for diagnosis.[6] Holo-transcobalamin (holo-TC), also called active vitamin B-12 due to its role in cobalamin transport into cells, has recently been proposed as a more effective marker for diagnosing B-12 deficiency.[7] Vitamin B-12 deficiency impacts the proper metabolism of folic acid, as the enzyme MTHFR is required to convert folate into its active form, N5-methyl-THF. This conversion is essential for processes like DNA synthesis, neurotransmitter production, and red blood cell formation. Therefore, sufficient vitamin B-12 levels are crucial for the proper metabolism of folic acid.[8]

The primary purpose of establishing reference intervals (RIs) for the blood levels of biochemical parameters is to define the range of normal physiological variation and to distinguish between healthy individuals and patients. RIs are crucial for accurately interpreting patient results during clinical assessments, offering essential guidance for diagnosis, treatment monitoring, and risk evaluation.

Corresponding Author: Dr Sedat Özdemir. Ankara Atatürk Sanatorium Training and Research Hospital, G blok Kat 1 Biyokimya Laboratuvarı Kuşcağız Mah. Sanatoryum Cad. No: 271 06280, Keçiören, Ankara, Turkey
Tel: (0312) 567 70 00
Email: drsedatozdemir@gmail.com, sedat.ozdemir@saglik.gov.tr
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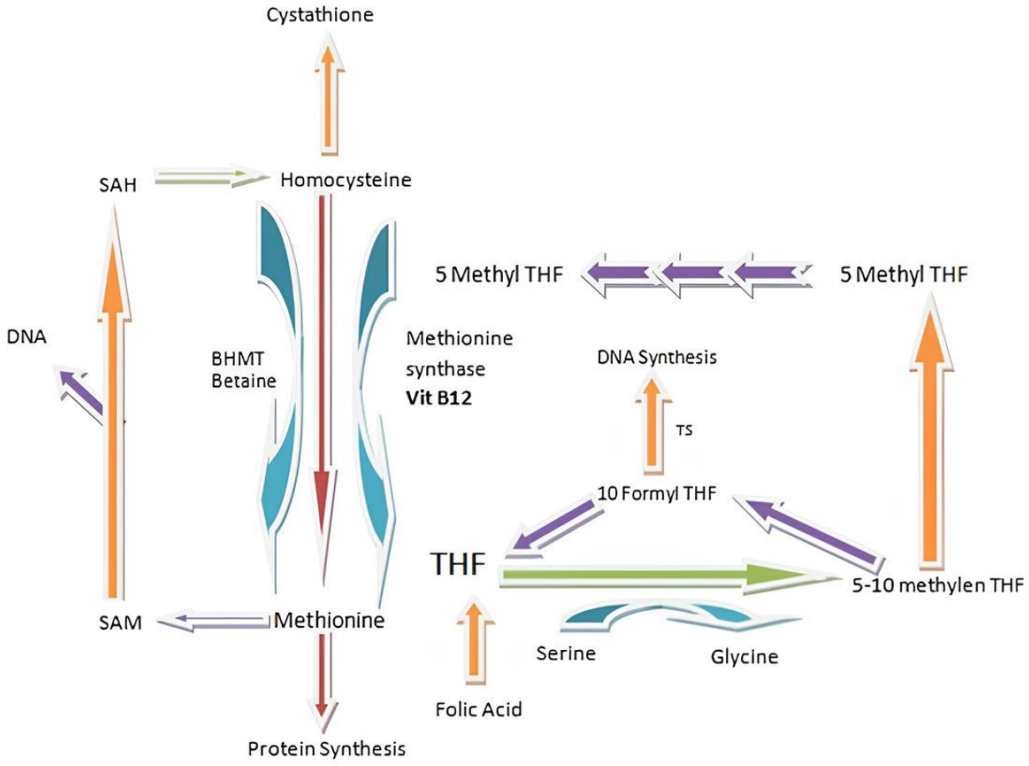

Figure 1. Formation of methionine via remethylation and role of vitamin B-12. TS: Thymidylate synthase, SAM: S-adenosyl methionine, SAH: S-adenosine homocysteine, BHMT: Betaine homocysteine methyl transferase, THF: Tetrahydrofolate, MTHFR: methylenetetrahydrofolate reductase

Tailored to the demographic and biological characteristics of the population, these intervals enhance the precision and reliability of clinical decision-making.

It should be emphasized that the RIs for assessing vitamin B-12 levels in this study should only be applied to older adults and not in younger and middle-aged individuals. Research suggests that, contrary to common belief, vitamin B-12 deficiency observed in older adults can also occur in younger and middle-aged individuals. Therefore, establishing appropriate reference intervals for this age group is essential for accurate diagnosis.

In the present study, we analyzed all biomarkers involved in vitamin B-12 metabolism, including vitamin B-12, holo-TC, homocysteine, folate, and plasma MMA, in healthy individuals aged 18–65 years. This study aimed to demonstrate that vitamin B-12 levels should be assessed not only through serum vitamin B-12 but also by using a panel of markers involved in vitamin B-12 metabolism. The present study also categorized young and middle-aged individuals (18–65 years) into several age groups to establish age-specific reference intervals for vitamin B-12 and related markers. This approach may help reveal how biomarkers change with age and the correlations between these biomarkers

METHODS

This study included healthy volunteers aged 18–65 years who visited the blood bank and outpatient clinics of Ufuk University, Rıdvan Ege Hospital. Blood samples were collected between October 2014 and May 2015 and were analyzed after obtaining informed consent from the volunteers. The study involved young and middle-aged healthy individuals who attended Ufuk University Hospital for routine check-ups or aesthetic procedures. We ensured that all participants were free from significant health conditions. Additionally, there are no conflicts of interest among the individuals who consented to participate in the study. Each group was analyzed based on sex and age range.

Vitamin B-12, folate, homocysteine, and holo-TC samples were collected in BD brand gel separator tubes (BD Vacutainer; Becton Dickinson, Meylan, France). Plasma MMA samples were collected in Li-heparin tubes (BD Vacutainer) for plasma separation. All samples were stored at −80°C until the day of analysis. At the biochemistry laboratory of Ufuk University, Dr. RıdvanEge Training and Research Hospital, serum vitamin B-12, holo-TC, homocysteine, and folate levels were measured using Abbott Architect i2000sr analyzer (Abbott Diagnostics, Abbott Park, IL, USA) based on the chemiluminescent microparticle immunoassay (CMIA). Periodic internal and external quality checks were performed to identify errors that could potentially affect the test results. Plasma MMA levels were measured using liquid chromatography–tandem mass spectrometry (LC–MS/MS) (Agilent Technologies®, Santa Clara, California, USA). The assay demonstrated a minimum detection limit of approximately 0.005 µM for plasma MMA. The analytical sensitivity of the LC-MS/MS system enabled precise and accurate measurement of MMA concentrations within this detection range, ensuring reliable evaluation of metabolic status. MMA, succinic acid, tert-butyl methyl ether, and hydrochloric acid n-butanol (3 M) were procured from Sigma-Aldrich (St. Louis, MO). Deuterium-labeled MMA (d3MMA) was purchased from CDN Isotopes (Quebec, Canada). ACS-grade methanol, phosphoric acid

(H3PO4), glacial acetic acid, and acetonitrile were obtained from Thermo Fisher Scientific (Pittsburgh, PA). Purified water (with18 MΩ resistance) was prepared using CLRW Clinical Laboratory Reagent Water Systems (Bergama Tıp, Izmir, Turkey) and was used for all samples, calibrators, and reagents. Quality control samples were analyzed at low-, medium-, and high-level classification. All samples were screened for MMA and spiked with MMA standard solution to achieve the desired concentrations.

The study included volunteers who were selected from healthy individuals visiting our hospital. Healthy individuals aged 18–65 years who had no systemic disease, were not on any medication including herbal or vitamin preparations, and had not received vitamin B-12 therapy over the last 6 months.

The study excluded participants who administered any medication affecting vitamin B-12 metabolism over the last 2 weeks (including those taking oral multivitamin tablets), women using oral contraceptives, individuals who had an acute infection over the last week, those with chronic diseases (systemic inflammatory diseases and liver and kidney diseases), pregnant women, women who gave birth in the last 3 months, those with low blood count and anemia criteria, those with positive hepatitis markers, and those who underwent surgery in the last 6 months.

Serum B12, folate, homocysteine, holotranscobalamin and serum MMA levels of the volunteers participating in the study were evaluated in accordance with CLSI EP09-A3 and IFCC-CLSI C28-A3 guidelines.

Ethics

The protocol for this study has been approved by the Ethics Committee of Ufuk University with the protocol number 27.12.2013/1. The research was conducted in compliance with the principles of the Declaration of Helsinki as revised in 1995 and 2000 in Edinburgh.

Statistical analysis

Statistical analysis was performed using IBM Statistical Package for Social Sciences (SPSS software v.21, Chicago, IL, USA), and reference interval analysis was performed using Reference Value Advisor v2.0 software program.[9-12] Numerical data were analyzed for normality of distribution using the Kolmogorov–Smirnov test. As the measured vitamin B-12 and all related markers were non-normally distributed, pair wise group comparisons were performed using the Mann–Whitney U test whereas multiple group comparisons were performed using the Kruskal–Wallis test. Intergroup comparisons were conducted using the Conover–Iman test. *p*-values of $p<0.05$ were considered to indicate statistical significance.

Also the study parameters were analyzed using the Mann–Whitney U test to determine if there were any differences in reference intervals between males and females.

RESULTS

A total of 6524 patients who visited the outpatient clinics of Ufuk University Dr. Rıdvan Ege Training and Research Hospital were considered for inclusion and selected based on the inclusion and exclusion criteria described in Methods. Overall, 460 patients were considered eligible. After face-to-face interviews, 438 volunteered to participate in the study. After sample review, 11 lipemic and 16 hemolyzed samples were excluded. Samples from seven volunteers could not be analyzed due to inadequate volume. The mean age of 404 volunteers who participated in the study was 25 (18-65) years. Among them, 156 (38.6%) volunteers were male and 248 (61.4%). There are no significant differences in vitamin B-12 ($p=0.899$) and its associated markers, including folate ($p=0.624$), homocysteine ($p=0.911$), Holo-TC ($p=0.534$), plasma MMA ($p=0.970$), between genders. Age and sex distribution of the volunteers, number of volunteers, and reference intervals by sex are presented in Table 1.

The present study revealed a RI of 139–619 pg/mL for vitamin B-12, 3.0–14.7 ng/mL for folate, 5.6–18.4 µmol/L for homocysteine, 10.7–101.4 pmol/L for holo-TC, and 0.01–0.8 µmol/L for plasma MMA (Figure 2).

Age-specific RIs for B-12 and related markers such as folate, holo-TC, and plasma MMA classified by age groups are shown in Table 2. However, RI for homocysteine did not vary by age. In regard to vitamin B-12, the age-specific RI was 132–630 pg/mL for young individuals aged 18–25 years (Group I), and 175–654 pg/mL for the oldest group aged 56–65 years (Group V). RI for folate was 2.7–11.4 ng/mL for Group I and 3.3–17.9 ng/mL for Group V. Regarding holo-TC, the lowest RI (8.9–88 pmol/L) was observed in Group II (26–35 years), whereas the highest RI (10.2–110 pmol/mL) was observed in Group IV (46–55 years). Plasma MMA levels did not vary by age, and the corresponding RI was found to be 0.01–0.8 µmol/L. The lowest RI for this parameter was 0.01–0.6 µmol/L, as observed in Group II (26–35 years)

Table 1. Age, gender and reference interval table

	95% RI		*p**	
	Men (n=156)	Women (n=248)		Total (n=404)
Age (Mean±SD)	36.9±16	29.9±13.3		32.6±14.8
Vitamin B-12 (pg/mL)	134-675	142-581	0.899	139-619
Folate (ng/mL)	2.7-14.9	3.2-13.6	0.624	3-14.7
Homocysteine (µmol/L)	5.8-23.2	5.4-17.4	0.911	5.6-18.4
Holo-TC (pmol/L)	9.4-97	10.3-102	0.534	10.7-101
Plasma MMA(µmol/L)	0.01-0.8	0.01-0.8	0.070	0.01-0.8

RI: reference intervals

†95% Reference interval were calculated according to the IFCC-CLSI C28-A3 guideline.

*$p < 0.05$ is considered statistically significant.

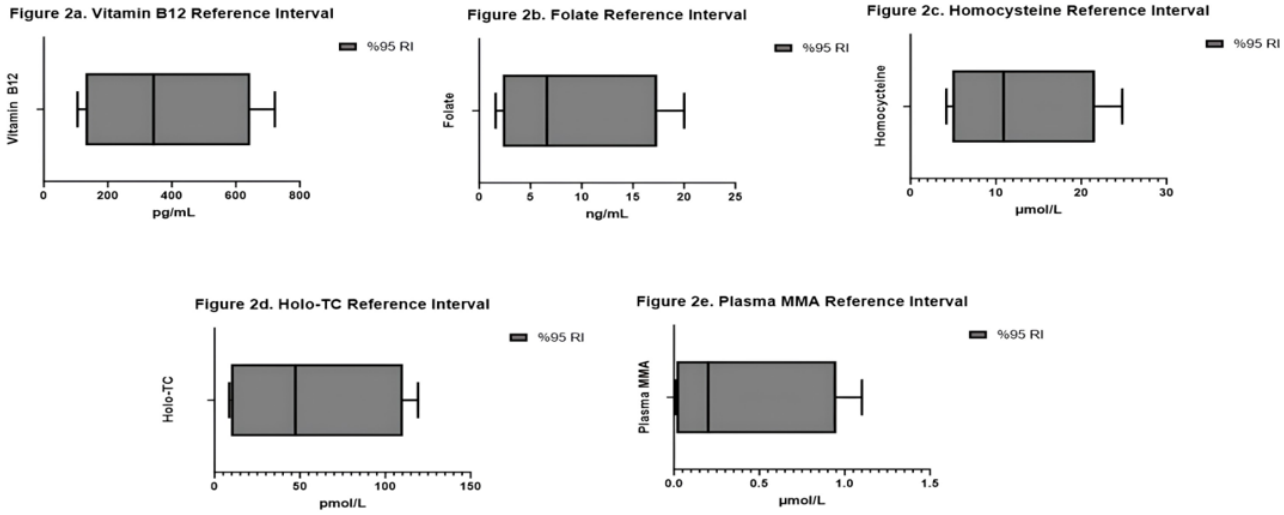

Figure 2. Our proposed 95% reference interval (RI) for serum (a) vitamin B12, (b) folate, (c) homocysteine, (d) holotranscobalamine (Holo-TC) and plasma (e) methylmalonic acid (MMA) based on blood samples from healthy individuals and patient data. Vitamin B12, folate and homocysteine were analyzed using chemiluminescent immunoassay; Holo-TC was analyzed using chemiluminescent microparticle immunoassasy; MMA was analyzed using LC-MS/MS method.

Table 2. Reference intervals by age groups

Age groups	2.5-97.5% RI†					
	Group I (18-25 y/o)	Group II (26-35 y/o)	Group III (36-45 y/o)	Group IV (46-55 y/o)	Group V (56-65 y/o)	Total (18-65 y/o)
n	211	59	37	39	58	404
Vitamin B-12 (pg/ml)	132-630	143-633	164-633	117-579	175-654	139-619
Folate (ng/ml)	2.7-11.4	2.3-18.3	3.1-18.6	3.2-13.7	3.2-17.9	3-14.7
Homocystein (µmol/L)	5.6-23	5.3-16.7	5.3-21.2	5.7-16.2	6.6-20.9	5.6-18.4
Holo TC (pmol/L)	10.5-101	8.9-88	9.4-102	10.2-110	8.3-115	10.7-101
Plasma MMA (µmol/L)	0.01-0.8	0.01-0.6	0.01-0.7	0.01-0.6	0.01-0.5	0.01-0.8

y/o: years old; RI: reference intervals.
†95% Reference interval were calculated according to the IFCC-CLSI C28-A3 guideline.

and Group IV (46–55 years).

Contrary to popular belief, there are age-related differences even among young to middle-aged healthy individuals when comparing Vitamin B-12 levels and other measured markers with different age groups.

In the present study, the correlation between parameters are examined. *p*-values and correlations among the tests are summarized in Table 3.

No correlation was found between homocysteine and plasma MMA (r = -0.03). Significant correlations were observed among other parameters (r > 0.05). Notably, a strong correlation was identified between vitamin B-12 and Holo-TC. Additionally, correlations were found between age and all parameters. A negative correlation was noted between age and plasma MMA (r = -0.256), while positive correlations were observed with other parameters.

DISCUSSION

The RIs determined in the present study for vitamin B-12, folate, homocysteine, and holo-TC were compared with those used in present laboratory. The RIs found in this study for all parameters were lower than the ones currently in use. The RI for plasma MMA was compared with the corresponding LC–MS/MS RI currently used in clinics, which showed that the current RI based on the analyzer was higher than the RI found in present study. This indicates that the use of international RIs for vitamin B-12 and associated markers may be inadequate for diagnosis and follow-up.

There were some limitations in the present study. As there have been few studies evaluating RIs for all parameters, we can only compare our results to only a small number of studies. A second limitation was that the enzymes methionine synthase and methylmalonyl-CoA mutase was not measured due to measurement difficulties and funding issues. Also, serum folate levels are considered an important biomarker for the short-term assessment of folate status. Serum folate reflects the immediate folate levels in the body and can be used for the early detection of folate deficiency. Studies have shown that low serum folate levels can reliably indicate folate deficiency. Additionally, the rise in serum folate levels following folate supplementation confirms its use as a reliable indicator of folate bioavailability and status in the body.[6] These findings support that serum folate levels alone can be a valid parameter for assessing folate status.

Serum vitamin B-12 is a commonly requested test in clinical practice, particularly for diagnosing deficiency in cases of neuropathic, hematologic, or cognitive symptoms. This highlights the importance of establishing a reliable RI for diagnosis, monitoring, and treatment.

Table 3. Correlation between parameters (r) and (*p*) values

Spearman's rho	Age	Vitamin B-12 (pg/ml)	Folate (ng/ml)	Homocysteine (µmol/L)	Holo TC (pmol/L)	Plasma MMA (µmol/L)
Age						
r	1.000	0.092*	0.198**	0.103*	0.126*	-0.256**
p		0.032	<0.0001	0.036	0.010	<0.0001
n	404	404	404	404	404	404
Vitamin B-12 (pg/mL)						
r	0.092*	1.000	0.362**	-0.478**	0.780**	-0.263**
p	0.032		<0.0001	<0.0001	<0.0001	<0.0001
n	404	404	404	404	404	404
Folate (ng/mL)						
r	0.198**	0.362**	1.000	-0.206**	0.336**	-0.184**
p	<0.0001	<0.0001		<0.0001	<0.0001	<0.0001
n	404	404	404	404	404	404
Homocysteine (µmol/L)						
r	0.103*	-0.478**	-0.206**	1.000	-0.372**	-0.003
p	0.036	<0.0001	<0.0001		<0.0001	0.952
n	404	404	404	404	404	404
Holo TC (pmol/L)						
r	0.126*	0.780**	0.336**	-0.372**	1.000	-0.198**
p	0.010	<0.0001	<0.0001	<0.0001		<0.0001
n	404	404	404	404	404	404
Plasma MMA (µmol/L)						
r	-0.256**	-0.263**	-0.184**	-0.003	-0.198**	1.000
p	<0.0001	<0.0001	<0.0001	0.952	<0.0001	
n	404	404	404	404	404	404

r: correlation coefficient
*: Correlation significant at the 0.05 level (2-tailed).
**: Correlation significant at the 0.01 level (2-tailed).

However, variations in analytical methods and instruments challenge the accuracy of diagnosis.

The HELENA Study included 1051, 12.5–17.9 years from 10 cities of 9 European countries and investigated vitamin B-12 levels using chemiluminescence via Siemens DPC Immulite 2000 autoanalyzer. According to the data obtained from this study, the RI for vitamin B-12 was 199.5–977.5 pg/mL (147.3–721.4 pmol/L).[13] Moreover, a study involving 302 healthy female university students aged 18–35 years in Australia, the RI for vitamin B-12 was determined to be 162.6–813 pg/mL (120–600 pmol/L) using chemiluminescence via Beckman Coulter UniCel DXI 800 Access autoanalyzer.[14] None of these RIs were consistent with those found in the present study. This may be due to the inclusion of only adolescents and the use of different analyzer in the HELENA study. Additionally, the Helena study is multicentered that may contribute to this difference. Andersen et al. investigated vitamin B-12 RIs in 129 individuals using Abbott Alinity analyzer via CMIA; vitamin B-12 RI was found to be 227.6–749.3 pg/mL (168–553 pmol/L),[15] which is similar to that found in this study. In another study, Demirin et al. assessed 1251 individuals aged 18–79 years and used Siemens DPC Immulite 2000 autoanalyzer, which yielded a reference interval of 158–563 pg/mL for vitamin B-12.[16]

Similar findings in Andersen et al.'s study may be attributed to the use of similar methods and reagents in both studies. In Demirin et al's study, they investigated a similar age group using a different analyzer and found a reference range similar to that found in the current study. This suggests that studies conducted with different age groups may give similar reference ranges regardless of device differences. In the National Health and Nutrition Examination Survey (NHANES), conducted with a large population, a value of 139 pg/mL was found for vitamin B-12, compared to the lower limit of 200 pg/mL (148 pmol/L) for vitamin B-12. However, this shows that regional factors also play an important role in vitamin B-12 deficiency.

Notably, this study found that vitamin B-12 levels were lower in younger individuals aged 18–25, primarily pursuing higher education, compared to those aged 56–65, classified as middle-aged. This may suggest a potential nutritional deficiency among the younger group engaged in academic activities.

The RI for folate identified in this study was lower than the range provided by the analyzer's manufacturer. This led us to the conclusion that the Turkish population is not only vitamin B-12 deficient, but also folate deficient. A retrospective study involving 723 Korean adults reported a RI of 2.9–38.0 ng/mL for folate.[17] Schwettmannn et al. conducted a study involving 144 volunteers using Abbott Architect i2000 analyzer and found a RI of 5.2–29.2 nmol/L (2.9–12.8 ng/mL) for folate,[18] whereas a study conducted by Önder et al. in 300 healthy individuals using Siemens Advia Centaur XP autoanalyzer revealed a RI for folate of 2.87–19.49 ng/mL.[19] Compared to the RI of 3–14.7 ng/mL for folate found in this study, it seems that the lower limits for folate were similar to the studies above regardless of the analyzer and method used for folate measurement. However, reference range found in Schwettmann et al. were the closest to this study; this suggests that age is an important determinant of folate levels. The comparison of RIs for folate in different age groups showed a significant difference between the

youngest and oldest groups. Contrary to popular belief, folate deficiency is observed in the younger population. Considering that folate is essential for fetal neurological development, especially during the reproductive period, subnormal folate levels among young individuals of reproductive age is a significant public health problem. Folate deficiency may be coupled with B-12 deficiency, indicating the importance of a combined assessment of serum levels of both vitamins.

Elevated homocysteine levels are a risk factor for coronary heart disease and are closely associated with low levels of vitamin B-12. The RI for homocysteine found in the present study was higher than that provided by the manufacturer, which indicates a significant vitamin B-12 deficiency and consequently elevated homocysteine levels. A study involving 20880 people revealed a homocysteine RI of 9–14.6 µmol/L.[20] The lower limit was higher, whereas the upper limit was lower than that found in the present study. Furthermore, the same study revealed a difference in RIs between males and females. However, no significant difference between males and females were found in this study. In a study conducted by in 3150 individuals, the RIs for homocysteine in females and males were 5.03–13.80 and 3.95–10.19 µmol/L, respectively.[21] The RIs reported in the study for both males and females were lower than those reported in our study. In males, homocysteine RI was reported to be 4.81–12.27 µmol/L for individuals aged 45–54 years and 5.00–14.80 µmol/L for those aged 55–64 years; in females, homocysteine RI was reported to be 3.74–8.98 µmol/L for those aged 45–54 years and 3.99–9.80 µmol/L for those aged 55–64 years. However, no significant difference were foundbetween males and females in the same age groups. RIs for those aged 46–55 years and 56–65 years were 3.9–16.1 µmol/L and 6.7–20.6 µmol/L, respectively. The RI for homocysteine found in the present study was higher than that reported by Kweon.[21] Furthermore, a study conducted by Lahiri et al. involving 1288 healthy individuals reported a homocysteine RI of 6.5–16.3 µmol/L,[22] which is similar to that found in present study. This suggests that differences in RIs may be due to diet and lifestyle rather than racial differences. In addition, as vitamin B-12 deficiency in the population leads to an increase in homocysteine levels, caution should be exercised in monitoring high homocysteine levels in populations with low vitamin B-12 levels.

There is scarce data in regards to RI for holo-TC. The present study found an interval of 10.7–101.4 pmol/L for holo-TC. Among the limited number of reports, a study recommended the measuring holo-TC levels to confirm subnormal B-12 levels and established a reference interval of 42–157 pmol/L.[23] Another study involving 105 healthy individuals aged 20–80 reported a holo-TC RI of 24–157 pmol/L.[24] Although the upper reference limits in both studies were the same, the lower reference limits were significantly different. In contrast, Al Aisari et al. revealed a holo-TC RI of 7.75–128 pmol/L.[25] Compared with the first two studies, our study revealed a considerably lower RI. The lower limit reported by Aisari et al. was lower than that reported in our study as well as other studies. However, the upper limit reported by Aisari et al. was higher than that reported in the present study. Holo-TC measurement maybe more suitable than total vitamin B-12 measurement for diagnosing vitamin B-12 deficiency.[7]

MMA is regarded as the most reliable marker for detecting intracellular vitamin B-12 deficiency. In cases of deficiency, impaired enzyme activity leads to MMA accumulation in the blood. Several studies have explored plasma MMA reference ranges using LC-MS/MS, with the present study identifying a range of 0.01–0.8 µmol/L. However, the NHANES study, which included 7300 volunteers, found that the plasma MMA range was 60–210 nmol/L (0.06–0.21 µmol/L).[26] Based on data from 10,020 individuals by reviewing the 2011–2014 NHANES data, Mineva et al. found that plasma MMA reference range was 70.6–451 nmol/L (0.076–0.45 µmol/L).[27] In another study involving 285 healthy individuals aged 18–69 years, the plasma MMA reference range was reported to be 0.10–0.40 µmol/L.[28] These three studies yielded results below the reference range found in this study. In contrast, a plasma MMA reference range of 0.15–0.782 µmol/L was reported by Obeid et al.[29] The upper limit of the reference range in the above-mentioned study is consistent with that reported in the present study, and the high plasma MMA level is due to the presence of individuals with low vitamin B-12 levels in the study. This suggests that the vitamin B-12 levels found in present study were low.

Relatively higher plasma MMA levels were found in this study compared to other studies, which may be attributed to B-12 deficiency in the population. The absence or reduced activity of enzymes caused by vitamin B-12 deficiency results in elevated plasma MMA levels, which may account for the higher reference range observed in this study.[30] Given the limited studies providing reference intervals for all analyzed parameters, we compared the RIs identified in this study with those reported in other publications separately.

Conclusions

The lack of standardization in vitamin B-12 measurement methods, combined with the potential for serum B-12 tests to produce falsely low or normal results, increases the risk of diagnostic errors.[3] Therefore, this study aimed to minimize diagnostic errors by establishing and presenting reliable reference ranges for vitamin B-12.

Altogether, these findings suggest that low levels of vitamin B-12 in the population may lead to low or high reference ranges for relevant markers. This study revealed that, contrary to popular belief, levels of vitamin B-12 and related markers were significantly lower not only in the older group but also in younger individuals, who are typically considered the most productive group engaged in higher education. Therefore, it is recommended that vitamin B-12 levels be evaluated alongside holo-TC levels, which show a strong correlation with B-12, in healthy young and middle-aged individuals to ensure accurate assessment. In addition, to obtain accurate results, the diagnosis of vitamin B-12 deficiency should be based on a panel that includes vitamin B-12 and related markers such as folate, homocysteine, holo-TC, and plasma MMA. Current methods cannot fully diagnose vitamin B-12 deficiency due to methodological limitations and the absence of standardized reference ranges. A comprehen-

sive panel, including relevant markers, may help diagnose subclinical cobalamin deficiency, which has been previously described and is thought to be common among young adults. Signs and symptoms such as poor academic performance, cognitive changes, difficulty understanding, and fatigue in young people should be taken seriously. It is essential to evaluate all vitamin B-12 related markers together to ensure appropriate treatment. Therefore, to accurately diagnose vitamin B-12 deficiency and provide effective treatment, it may be beneficial to assess all relevant markers collectively.

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CONFLICT OF INTEREST AND FUNDING DISCLOSURE

The authors declare no conflict of interests.

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