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Original Research Article

B12 deficiency epidemic? Should B12 level lower limit reference values be decreased?

Keramettin Yanık[1], Atakan Özkan[2]*

[1]Special Esencan Hospital Laboratory, Istanbul, Turkey
[2]Department of General Surgery, Special Esencan Hospital, Istanbul, Turkey

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***Correspondence:**
Dr. Atakan Özkan,
E-mail: atakancerrah34@gmail.com

ABSTRACT

Background: This study aims to examine B12 levels in general in our society, to determine the rates of B12 deficiency, and to determine and recommend new B12 reference range values according to current data.
Methods: A total of 16497 patients who were admitted to our secondary care hospital for various reasons in the three-year period between January 2021 and December 2023 and whose B12 levels were tested in their serum were included in the study. B12 levels of all patients were measured in the hospital biochemistry laboratory on Architect i1000SR and i2000 SR (Abbott diagnostics, Ireland) devices.
Results: The reference ranges for B12 levels, determined as 2.5% and 97.5%, were 130-1084 pg/ml for adult men, and as 137-993 pg/ml for adult women. Considering 200 pg/ml as the normal lower limit for B12 level, low levels were found in 14.6% of all patients.
Conclusions: Our study is one of the rare studies in which B12 reference ranges are investigated up-to-date with a large patient participation. Our findings showed that the rate of B12 deficiency in the society is very high compared to the generally accepted reference lower limit values, that B12 levels in the society are very low compared to these reference values, and perhaps that the reference lower limit values may need to be reduced to 149-138 pg/ml in individuals under 18 years of age, 130-137 pg/ml in adults, and 123-125 pg/ml in the elderly.

Keywords: B12, B12 deficiency, Cobalamin, Reference values, Lower limit

INTRODUCTION

Vitamin B12 is a water-soluble vitamin that is critical for the human body. Body cannot produce this vitamin, also known biochemically as cobalamin, so it must be obtained from external sources. Essentially, vitamin B12 acts as a catalyst in a number of biological processes such as DNA synthesis, neurotransmitter production and myelin sheath formation. Vitamin B12 is usually found in animal sources such as meat, fish and dairy products. Vitamin B12 deficiency can lead to nervous system disorders, megaloblastic anemia and other serious health problems.[1-6]

Three transport proteins, intrinsic factor (IF), haptocorrin (HC), and transcobalamin (TC), along with their respective membrane receptors have role in absorbtion of vitamin B12. The primary cause of vitamin B12 deficiency is often insufficient dietary intake in vegans, while malabsorption issues are linked to digestive diseases. The stomach releases B12 from food carrier proteins, binding it to HC. Degradation by pancreatic proteases and pH changes facilitate transfer of B12 to IF in duodenum. Cubilin and amnionless, components of the receptor, mediate B12 uptake in distal ileum. Bile excretes some liver B12, which undergoes enterohepatic circulation. Causes of B12 malabsorption encompass 5 inherited disorders, such as IF deficiency and Imerslund-Gräsbeck disease, as well as conditions like Addison's pernicious anemia, obesity, bariatric surgery, and gastrectomies. Other contributing factors involve pancreatic insufficiency, obstructive jaundice, tropical

sprue, celiac disease, bacterial overgrowth, parasitic infestations, Zollinger-Ellison syndrome, inflammatory bowel diseases, chronic radiation enteritis of the distal ileum, and short bowel. Assessment of B12 deficiency is recommended in bariatric surgery follow-ups, and genetic causes are likely underestimated in adult cases.[1,2,7]

B12 deficiency is common in society. If symptoms or findings such as anemia and forgetfulness are present, patients are treated for B12 deficiency. However, a significant portion of patients with low B12 may not have any clinical symptoms.[8-11] Some studies have shown that B12 levels in society are generally low, and in some studies, reference ranges can be changed.[12-21] This study aims to examine B12 levels in general in our society, to determine the rates of B12 deficiency, and to determine and recommend new B12 reference range values according to current data.

METHODS

Patients and tests

A total of 16,497 patients who were admitted to our secondary care hospital for various reasons in the three-year period between January 2021 and December 2023 and whose B12 levels were tested in their serum were included in the study. Patients with diagnoses related to B12 deficiency symptoms, those with anemia, patients with neurological symptoms, patients with significantly abnormal values in the complete blood count, those diagnosed with blood diseases, oncology patients, those with chronic diseases that may affect B12 levels, those with severe clinical conditions, those with urgent reasons Those who applied to the emergency department and intensive care unit patients were not included in the study. Additionally, the results of samples that caused repeat testing were not included in the study. Age and gender information and B12 test results of the patients were obtained retrospectively from hospital records.

B12 levels of all patients were measured in the hospital biochemistry laboratory on Architect i1000SR and i2000 SR (Abbott diagnostics, Ireland) devices. The process of collecting, transferring and testing samples was carried out in accordance with the recommendations of the manufacturer companies.

Statistical analysis

All statistical analyzes in the study were performed using SPSS 25.0 software (IBM SPSS, Chicago, IL, USA). Distributions of nominal or ordinal variables were given as numbers and percentages. Descriptive data are shown as mean, standard deviation and percentiles. Reference ranges were accepted as 2.5% and 97.5% percentile values. Since the highest value that could be measured in the device was 2000 pg/ml, all possible higher values were accepted as 2000 and the mean and standard deviation values were calculated in this way.

RESULTS

The mean age of the patients was 37.5±18.9 (range 0-99) and 10818 (65.6%) were women. A total of 2177 (13.2%) of the patients were under 18 years of age, and 14320 (86.8%) were adults. The clinics from which the most samples came were internal medicine (32.9%), general surgery (17.8%), dermatology (11.3%) and pediatrics (10.7%) (Table 1).

Table 1: Distributions according to some variables.

Variables	N	Percentage (%)
Total	16497	100
Gender		
Male	5679	34.4
Female	10818	65.6
Age groups (in years)		
<18	2177	13.2
18 +	14320	86.8
Age groups (in years)		
<1	82	0.5
1-5	932	5.6
6-11	635	3.8
12-17	528	3.2
18-44	9021	54.7
45-64	3746	22.7
>65	1553	9.4
Year		
2021	5496	33.3
2022	6110	37.0
2023	4891	29.7
Clinics		
Internal diseases	5432	32.9
General surgery	2941	17.8
Dermatology	1863	11.3
Pediatry	1769	10.7
For check up	1622	9.8
Gynecology and obstetrics	801	4.9
Emergency room	1126	6.8
Physical medicine and rehabilitation	345	2.1
Cardiology	116	0.7
Chest diseases	82	0.5
Ear nose throat	65	0.4
Nutrition unit	64	0.4
Exam for job application	63	0.4
Neurochirurgy	58	0.4
Orthopedics	49	0.3
Urology	40	0.2
Other	61	0.4

The mean B12 level in men under 18 years of age is 439.0±236.6 pg/ml; for those over 18 years of age, it was 382.4±246.5 pg/ml. The mean B12 level in women under the age of 18 is 436.1±242.3 pg/ml; for those over 18 years of age, it was 380.4±231.5 pg/ml (Table 2).

The reference ranges for B12 levels, determined as 2.5% and 97.5%, were 149-1018 pg/ml for men under the age of 18 and 130-1084 pg/ml for those over the age of 18. For women, reference ranges were determined as 138-1085 pg/ml under the age of 18 as weel as 137-993 pg/ml over the age of 18. In addition, reference value ranges for men over 65 years of age were determined as 123-1482 pg/ml for men and 125-1319 pg/ml for women.

Reference ranges and percentiles determined for each age group shown in Table 3.

Considering 200 pg/ml as the normal lower limit for B12 level, low levels were found in 14.6% of all patients. According to this limit value, the rate of low B12 patients was 9.6% under the age of 18, and 15% in all adults over the age of 18, and 16.1% for those over 65 years of age. When the lower limit was accepted as 150 pg/ml, the rate of patients with low B12 levels was determined as 4.1%. According to this limit value, the rate of patients with low B12 levels was 3.0% under the age of 18; 4.3% among all adults over 18 years of age; and 5.3% for those over 65 years of age (Table 4).

Table 2: Mean B12 serum levels by gender and age groups (pg/ml).

Variables	Gender					
	Male			Female		
	N	Mean	SD	N	Mean	SD
Age group (in years)						
<18	1051	439.0	236.6	1126	436.1	242.3
18 +	4628	382.4	246.5	9692	380.4	231.5
Age group (in years)						
<1	46	421.8	196.0	36	393.1	177.8
1-5	492	467.2	251.8	440	471.8	270.1
6-11	306	449.9	216.6	329	473.4	223.1
12-17	207	360.0	218.8	321	353.8	204.3
18-44	2870	372.8	235.6	6151	361.4	203.9
45-64	1245	382.0	227.3	2501	407.9	252.2
>65	513	436.6	330.2	1040	426.4	307.5

Table 3: B_{12} percentile values according to gender and age groups (pg/ml).

Percentiles	General	Age (in years)								
		<18	18 +	<1	1-5	6-11	12-17	18-44	45-64	65+
Male age groups, (n=5679)										
Min	50	75	50	133	75	127	108	50	77	50
2.5	133	149	130	169	142	165	137	133	128	123
5	156	176	152	170	178	197	153	154	151	143
10	183	205	179	186	205	222	185	177	186	177
25	241	275	234	276	285	286	232	229	242	242
50	328	385	314	376	411	403	321	308	322	333
75	473	550	454	618	589	565	398	442	460	506
90	669	723	648	715	773	699	563	642	635	820
95	844	886	833	765	928	883	712	795	796	1177
97.5	1066	1018	1084	824	1118	1046	907	1001	981	1482
Max*	2000	2000	2000	898	1941	1308	2000	2000	2000	2000
Female age groups, (n=10818)										
Min	47	71	47	112	92	132	71	50	50	47
2.5	137	138	137	112	132	207	122	137	140	125
5	155	168	155	147	163	222	149	154	160	149
10	182	200	181	186	197	247	178	179	190	176
25	239	274	236	276	290	316	230	231	248	241
50	328	377	323	384	415	423	308	314	344	340
75	459	534	450	476	576	572	419	430	484	503
90	642	735	627	669	804	783	560	590	686	740
95	811	919	795	795	997	973	695	726	883	1069
97.5	1009	1085	993	824	1215	1067	842	890	1106	1319
Max*	2000	1878	2000	832	1878	1487	1699	2000	2000	2000

*The maximum measured value can be 2000 pg/ml. Values above 2000 are considered 2000. Min: Minimum, max: Maximum.

Table 4: Low B12 level distributions according to limit values.

Variables	<200 pg/ml		<150 pg/ml	
	N	%	N	%
General	2411	14.6	683	4.1
Age group (in years)				
<18	210	9.6	65	3.0
>18 (all adults)	2201	15.4	618	4.3
Age group (in years)				
18-44	1469	16.3	388	4.3
45-64	482	12.9	148	4.0
>65	250	16.1	82	5.3

DISCUSSION

Low B12 can lead to a variety of complications depending on its level and duration. In order to determine treatment management, serum levels of B12 or its metabolites must be determined in patients. However, the fact that very different reference ranges are used for B12 serum levels among automated systems, in clinical practice, and in research causes variable results in the diagnosis of B12 deficiency.[1-5] In our study, current reference value ranges for B12 levels were investigated for each age group with a large number of participants.

To measure B12 deficiency, in addition to directly testing the B12 serum level, B12 metabolites homocysteine or methylmalonic acid levels can also be measured. However, apart from the level tests determined in this laboratory regarding B12, there is still no gold standard method or clinically definitive finding today.[22] In B12 deficiency, megaloblastic anemia may develop depending on the severity and duration of the deficiency, as well as neurological symptoms such as forgetfulness, neuropathy, sensory loss and balance problems, gastrointestinal symptoms such as glossitis, stomatitis and diarrhea, some cardiovascular diseases and mental problems due to the increase in homocysteine levels. Depression and anxiety may be observed.[1-5,23] It has been clinically stated that vitamin B12 has an effect on the healing of surgical wounds and that B12 deficiency causes a delay in wound healing.[24-28] However, a significant portion of patients do not show clinical symptoms.[1-5] One of the most commonly used B12 lower limit reference values is 203 pg/ml (150 pmol/ml).[17-22] The lower limit of B12 level in adults was determined by Bolaman et al 160 pg/ml, Seal et al 203 pg/ml, Eussen et al 271 pg/ml, Castelli et al accepted different values such as 350 pg/ml.[13-16] In some studies, similar to our study, B12 levels of many participants were measured and reference ranges were investigated according to 2.5% and 97.5% percentile values. For example, Abildgaard et al determined the lower limit reference value of B12 in the 18-45 age range as 174 pg/ml.[20] Andersen et al in their study with 34 thousand patients in Denmark using three separate automated systems, they determined the B12 lower limit values as 133 pg/ml, 172 pg/ml and 182 pg/ml.[18] Jassam et al found the lower limit reference values in adults as 190 pg/ml, 181 pg/ml and 110 pg/ml in their study with three separate automated devices.[19] Schwettmann et al found the lower limit value to be 180 pg/ml in their study, in which they used the same device as in our study.[21] In our study, the lower limit value for B12 levels, determined as 2.5% percentile, was found to be 130 pg/ml in men over the age of 18, and the lower limit value in women was 137 pg/ml over the age of 18. Additionally, in our study, when 200 pg/ml was accepted as the normal lower limit for B12 level, it was found that 15.6% of all adults had low levels. When the lower limit is accepted as 150 pg/ml, the rate of adult patients with low B12 levels is determined as 4.3%. In our study, the presence of a clinical symptom for B12 deficiency could not be taken as a reference. Therefore, these findings show that (i) these values between 130-149 should be taken instead of the generally and widely accepted B12 reference lower limit value of 200 pg/ml, or (ii) the B12 level in the society is generally low. Compared to the commonly accepted lower limit value, the rate of 14.6% of patients with low B12 levels obtained in our study is a very high value. When determining reference ranges, taking 2.5% percentile values as the lower limit of the normal value shows that the expected rate of patients with low levels for that marker in society is 2.5%. According to this assumption, the findings obtained from our study may indicate that the lower normal limit value should be lowered, rather than the general low B12 level in the society.

It has been stated that B12 levels decrease with age and are generally higher in children.[1-4,29] Abildgaard et al in their study in Denmark in 2022, they determined the lower limit reference value between the ages of 12 and 18 as 199 pg/ml.[20] In our study, the lower limit value for B12 levels, determined as 2.5% percentile, was found to be 149 pg/ml in men under the age of 18, and the lower limit value in women was 138 pg/ml under the age of 18. In our study, when the lower limit value is accepted as 200 pg/ml, the rate of B12 deficiency in children is 9.6%; When 150 pg/ml was accepted, it was found to be 3.0%. All these findings show that, as in adults, the incidence of low B12 in children is either very high in general, or the reference lower limit value should be reduced.

It has been stated that B12 serum levels decrease with age and, accordingly, the incidence of B12 deficiency is

higher in the elderly.[22,29] Many studies did not use a separate reference range for the elderly. As the reference range used increased, the prevalence of B12 deficiency was found to be higher in the elderly. For example, in older studies, the lower limit value was taken as 350 pg/ml and the rate of B12 deficiency could reach up to 40% in the elderly.[12,22] Abildgaard et al tried to determine separate reference value ranges according to age groups and found the lower limit reference value of B12 over the age of 45 to be 176 pg/ml.[20] In our study, the lower limit reference value for men over 65 years of age was determined as 123 pg/ml in men and 125 pg/ml in women. In our study, when 200 pg/ml was accepted as the normal lower limit for B12 level, the rate of patients with low B12 levels was found to be 16.1% over the age of 65, and when 150 pg/ml was accepted as the lower limit, it was 4.1%. All these findings support that B12 levels decrease in the elderly and show that, as in other age groups, the rate of patients with low B12 levels is either very high in the elderly or the B12 lower limit reference value should be reduced.

The fact that a reference symptom that could clinically detect B12 deficiency (and/or elevation) was not used in our study is a limitation that prevents us from clinically determining the reference range. However, such a reference method has not been used in the literature. The high number of patients in our study is a factor that positively affects the reliability of using the obtained values as reference values.

CONCLUSION

Our study is one of the rare studies in which B12 reference ranges are investigated up-to-date with a large patient participation. The findings obtained from our study showed that the rate of B12 deficiency in the society is very high compared to the generally accepted reference lower limit values, B12 levels in the society are very low compared to these reference values, and perhaps the reference lower limit values may need to be reduced. Our findings suggested that, depending on gender, the lower limit may need to be reduced to 149-138 pg/ml in individuals under 18 years of age, 130-137 pg/ml in adults, and 123-125 pg/ml in the elderly.

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