ORIGINAL RESEARCH

Bagcilar Med Bull 2020;5(4):160-165
DOI: 10.4274/BMB.galenos.2020.07.031

Determination of Reference Range for B_{12} and Folate Levels According to Laboratory Data in an Adult Population

Yetişkin Bir Popülasyonda Laboratuvar Verilerine Göre B_{12} ve Folat Düzeylerinin Referans Aralığının Belirlenmesi

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Abstract

Objective: The aim of this study was to establish reference intervals (RIs) according to laboratory data in a population and to assess the vitamin B_{12} (B_{12}) and folate status related to RIs for all age and gender groups.

Method: The data were retrospectively provided from the laboratory information system of Balıkesir State Hospital. The selected patient population comprised 36.284 individuals (70.2% female, 29.8% male) between the age of 18 and 80 years. These participants were separated into six age-based subgroups (18-30, 31-40, 41-50, 51-60, 61-70 and 71-80 years). B_{12} and folate concentrations were measured by the ARCHITECT i2000sr (Abbott Diagnostics, Abbott Park, IL, USA) autoanalyzer. Extreme values were excluded by using IBM SPSS. The 95% RIs were obtained by the non-parametric method.

Results: The results of 20.850 patients for B_{12} and of 14.183 for folate were evaluated. The mean ages of patients for B_{12} and folate were 48.9±16.3 years and 49.7±16.6 years, respectively. Mean ± standard deviation concentrations of B_{12} and folate were 220±77 pmol/L and 13.9±6.5 nmol/L, respectively. 95% RIs were calculated as 97-397 pmol/L for vitamin B_{12} and 5.17-30.9 pmol/L for folate in the entire population. There are statistically significant differences between women and men for B_{12} and folate. In addition, there is a significant difference between age groups for folate but not for B_{12} concentrations.

Conclusion: In this study, differences between the reference ranges recommended by the manufacturer and the reference ranges of our own population were found. Our results indicate that determining the true reference range is vital.

Keywords: Folate, laboratory data, reference range, Turkey, vitamin B_{12}

Öz

Amaç: Bu çalışmanın amacı, bir popülasyondaki laboratuvar verilerine göre referans aralıklar (RIs) oluşturmak ve tüm yaş ve cinsiyet grupları için RIs ile ilişkili olarak vitamin B_{12} (B_{12}) ve folat durumunu değerlendirmektir.

Yöntem: Laboratuvar datası Balıkesir Devlet Hastanesi laboratuvar bilgi yönetim sisteminden retrospektif olarak elde edildi. On sekiz-80 yaş arası 36,284 hasta (kadın için %70,2, erkek için %29,8) seçildi. Data, altı alt yaş grubuna ayrıldı (18-30, 31-40, 41-50, 51-60, 61-70 ve 71-80 yaş). B_{12} ve folat konsantrasyonları ARCHITECT i2000sr (Abbott Diagnostics, Abbott Park, IL, ABD) otoanalizörü ile ölçüldü. Uç değerler IBM SPSS kullanılarak atıldı. RIs %95 dağılımda non-parametrik indirekt yöntem kullanılarak hesaplandı.

Bulgular: B_{12} için 20,850 ve folat için 14,183 hastanın sonuçları değerlendirildi. B_{12} ve folat hastalarının yaş ortalaması sırasıyla 48,9±16,3 ve 49,7±16,6 idi. B_{12} ve folatın ortalama ± standart sapma konsantrasyonları sırasıyla 220±77 pmol/L ve 13,9±6,5 nmol/L idi. Tüm popülasyon için %95 RIs, B_{12} vitamini için 97-397 pmol/L ve folik asit için 5,17-30,9 pmol/L olarak hesaplandı. B_{12} ve folat için kadın ve erkek arasında istatistiksel olarak anlamlı fark vardı. Folat için RIs yaş grupları arasında anlamlı bir fark vardı. Ancak B_{12} konsantrasyonları için önemli bir fark yoktu.

Sonuç: Bu çalışmada imalatçı tarafından önerilen RIs ile kendi popülasyonumuzun RIs'leri arasında fark bulunmuştur. Sonuçlarımız, her laboratuvarın kendi referans aralığını belirlemesinin önemli olduğunu göstermektedir.

Anahtar kelimeler: B_{12} vitamini, folat, laboratuvar verileri, referans aralığı, Türkiye

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Cite this article as: Alpdemir M, Alpdemir MF. Determination of Reference Range for B_{12} and Folate Levels According to Laboratory Data in an Adult Population. Bagcilar Med Bull 2020;5(4):160-165

Introduction

The deficiency of vitamin B_{12} is a severe nutritional problem all over the world, as subclinical deficiency can impact well-defined risk groups. However, vitamin B_{12} deficiency is foremost of prevalent vitamin deficiencies. There is no general agreement on cut-off level for vitamin B_{12} and its co-markers, including folate, methylmalonic acid, holotranscobalamin, and homocysteine (1).

A reference interval is defined as the interval that detects the reference values for medical laboratory tests, provided from the sample reference distribution of the values obtained from well-described healthy people with particular statistical methods (2). The both Clinical and Laboratory Standards Institute (CLSI) and Clinical and International Federation of Clinical Chemistry (IFCC) recommend that each professional laboratory should detect its own reference intervals (RIs) (2,3). Owing to laboratory and regional differences based on population, technical instruments, nutrition, and selection of the reference people, it is imperative for most of medical laboratories to detect their own RIs (1-4).

In estimating the RIs, there are direct and indirect methods to distinguish the reference groups that mostly represent the characteristic features of population. The direct method involves the selection of reference people from the major survey group according to pre-described standards. After this survey was completed, laboratory tests of the participants are measured (1). The indirect method, by contrast, involves the choice of test results in accordance with specific criteria from laboratory data in which the examination of the results is saved regardless of individuals' features (4).

RIs for these vitamins can vary significantly among populations. The aim of this study was to demonstrate RIs in a population and to assess the folate and vitamin B_{12} status related to RIs.

Materials and Methods

Study Group

The results were obtained retrospectively from the laboratory information system of Balıkesir State Hospital between the years of 2017 and 2019. The data used were approved by the hospital administration. This study was approved by the clinical research ethical committee of Medicine Faculty of Balıkesir University. The data of 36.284 patients (70.2% female, 29.8% male) between the ages of 18 and 80 years were selected after extreme values were excluded. The age groups of the patients were separated into six subgroups (18-30, 31-40, 41-50, 51-60, 61-70, and 71-80 years). The results of patients from intensive care units and inpatient clinics were excluded from the study. Any patients who had a vitamin deficiency, megaloblastic anemia, malignancy or chronic disease were also eliminated. In order to avoid duplicated results and prevent the interference of patients taking vitamin supplements, only the initial vitamin B_{12} and folate values of the subjects were included. Vitamin B_{12} and folic deficiencies were defined as low, <148 pmol/L for vitamin B_{12} and <6.8 nmol for folate (5).

Analytical Method

B_{12} and folate concentrations were measured by ARCHITECT i2000sr (Abbott Diagnostics, Abbott Park, IL, USA) autoanalyzer. For the internal quality control (IQC) of IQC material (three levels), the manufacturer was used. The analytical test performance which pertains to intra-assay coefficients of variability (CVs), inter-assay CVs, and total analytical error is shown in Table 1. The external quality assessment scheme (EQAS) results were evaluated in the EQAS immune-assay monthly program from BIO-RAD laboratories (BIO-RAD laboratories, Diagnostic group, California, USA) for the all periods included in the study. EQAS average CV and Z-score were 6.5% and 0.29 for vitamin B_{12} and were 8.5% and 0.29 for folate. Patient samples were collected into serum separator tubes for B_{12} and folate. The

Table 1. The analytical performances of B_{12} and folate

		Concentrations	Intraassay CVs (%)	Interassay CVs (%)	TAE
B_{12}, pmol/L	Level 1	191	5.3	7.3	11.85
	Level 2	378	4.8	6.0	
	Level 3	700	4.8	5.2	
Folate, nmol/L	Level 1	8.9	5.2	6.1	11.49
	Level 2	17.0	3.8	5.1	
	Level 3	34.1	3.1	3.4	

CV: Coefficients of variability, TAE: Total analytical error

samples were freshly analyzed on the same day. Samples with hemolysis, lipemia, or icterus were excluded.

Statistical Analysis

Extreme values were excluded by using IBM SPSS. The central 95% RIs were calculated using indirect non-parametric method. The results were expressed in the form of mean ± standard deviation (SD) and percentages (%). The normal distribution was tested by the Kolmogorov-Smirnov Z-test. According to the results from the Kolmogorov-Smirnov Z-test, the RIs of the groups were estimated by parametric or non-parametric methods in accordance with the IFCC recommendations. The distribution was detected to be non-parametric by the Kolmogorov-Smirnov Z-test. Upper and lower limit values of the RIs were calculated using the non-parametric method (percentile estimation method), and points corresponding to 95% of the distribution were sought (3). Confidence intervals of 90% of references' limit values were estimated according to guidelines (3).

The Mann-Whitney U test was used to compare gender groups, while a One-Way variance analysis was used to compare age subgroups. For multiple comparisons of groups that showed differences in variance analysis, the Tukey test was used for groups that had homogeneous variance, and the Tamhane test was used for groups that did not have homogeneous variance. P<0.05 was accepted as statistically significant.

Results

The results of 20.850 patients for B_{12} and 14.183 for folate were evaluated, after eliminating patients that had an exclusion criterion and extreme values. The mean ± (SD) and percentages according to gender for analyses conducted after the exclusion of extreme values are shown in Table 2.

The mean ages of patients for B_{12} and folate were 48.9±16.3 and 49.7±16.6 years, respectively. In total, the mean concentrations of B_{12} and folate were 222±77 pmol/L and 13.9±6.5 nmol/L, respectively. 95% RIs were calculated as 97-397 pmol/L for vitamin B_{12} and 5.17-30.9 pmol/L for folate for the entire population. There were statistically significant differences between women and men for B_{12} and folate. There was a significant difference among the age groups for folate, but no significant difference among the age groups for B_{12} concentrations. RIs of vitamin B_{12} and folate for age groups are shown in Tables 3 and 4.

Out of the entire study population, 17.8% had serum vitamin B_{12} concentrations <148 pmol/L and 8.5% had <6.8 nmol/L concentrations of the serum folate. The prevalence of vitamin B_{12} and folate deficiencies for gender and age groups are presented in Table 5.

Table 2. RIs for serum folic acid and vitamin B_{12} according to laboratory data in an adult population

		n (%)	Ages (Mean ± SD)	Mean ± SD	RIs Lower-upper (90%CI)	Manufacture's RIs
B_{12}, pmol/L	Female	14.736 (70.7)	47.8±16.1	222±77	107-397 (357-436)	138-654*
	Male	6.114 (29.3)	51.4±16.7	216±78	93-396	-
Folate, nmol/L	Female	10.254 (72.3)	48.7±16.1	14.4±6.7	5.2-33.2	7.0-43.4*
	Male	3.929 (27.7)	51.5±17.1	12.7±5.9	5.9-29.3	-

*: There is not a RIs according to gender. N: Number of patients, SD: Standard deviation, RIs: Reference intervals, CI: Confidence internal

Table 3. RIs for serum vitamin B_{12} according to age subgroups

Groups	n (%)	Ages (Mean ± SD)	B_{12}, pmol/L Mean ± SD	RIs	
				Lower (90% CI)	Upper (90% CI)
18-30	2.552/888	23.1±3.7	218±70	104 (97-114)	383 (345-421)
31-40	2.439/787	35.7±2.8	220±75	103 (93-113)	393 (354-432)
41-50	3.145/1.017	45.7±2.7	220±75	102 (92-112)	393 (354-432)
51-60	3.032/1.310	55.1±2.9	222±78	98 (88-108)	400 (360-440)
61-70	2.241/1.256	65.0±2.8	217±83	90 (81-99)	403 (363-443)
71-80	1.327/856	74.9±2.73	210±84	83 (75-91)	409 (368-450)
All	14.736/6.114	48.9±16.3	220±77	97 (87-107)	397 (357-436)

n: Number of female/male patients, SD: Standard deviation, CI: Confidence internal

Discussion

Presently, clinical laboratories use RIs frequently, a practice which is advised by the manufacturer. However, these RIs are not always representative of the members of the population in question (3). Thus, the IFCC and the CLSI recommend that each laboratory determines its own RIs. Each laboratory should examine the transferability of the expected values to its own patient population and determine its own reference interval (2,3). We identified the serum RIs for folate and vitamin B_{12} according to big laboratory data in an adult population. In this study, differences were found between the RIs recommended by the manufacturer and those determined by our own population. There was not a high prevalence for deficiency of folate and vitamin B_{12} in our population.

In this study, we determined RIs 97-397 pmol/L according to the total population for vitamin B_{12}. These values were lower than the upper and lower limits recommended by the manufacturer. In the conducted studies in our country, the RIs for vitamin B_{12}, the lower limit varies between 70 and 235 pmol/L, and the upper limit varies between 374 and 1.474 pmol/L (6-9). In the study of Bakan et al. (6), RIs were determined between 70 and 368 pmol/L in the Erzurum region, which has a lower socioeconomic status and is located in the east of our country. In the study of Oncel et al. (10), RIs were determined between 75 and 518 pmol/L in the Konya region, which has a lower socioeconomic status and is located in the middle of our country. In the study of Köseoğlu et al. (7), RIs were determined between 142 and 953 pmol/L in the Izmir region, which has a higher socioeconomic status and is located in the west of our country. Avcı and Aslan (11) determined RIs as 106-393 pmol/L for the entire population in a study with non-parametric methods using laboratory data similar to our study. The determined RIs in our study are similar to the values determined by Avcı and Aslan (11). In our study, for the RIs of vitamin B_{12}, there was a significant difference between genders.

The RIs determined according to the total population in this study were 5.2-30.9 nmol/L for folate. In the study conducted in Istanbul in our country, a direct method was used to determine RIs between 5.1 and 47 nmol/L (7). In a study conducted with a direct method in Izmir, the determined RIs were between 12.7 and 45.3 nmol/L (7). In a study conducted by Bakan et al. (6), the RIs determined with the direct parametric method were between 9 and 33 nmol/L for women and 9 and 28 nmol/L for men. In the study conducted with the direct method by Tanyalcin et al. (12), the determined RIs were between 8.9 and 41.1 nmol/L for women and 5.7 and 39.9 nmol/L for men. As evidenced by these two studies, RIs for folate were lower in men than in women. These results are similar to our results: both suggest that RIs differ according to gender (6,8,11).

Deficiency of vitamin B_{12} is a severe situation for health all over the world. It correlates with insufficient nutrition, which often results from socioeconomic conditions and increased vegetarian eating habits. Vitamin B_{12} and folate deficiencies have long been well-known to cause adverse effects on health, such as neuropathy and anemia (13). In this study, the most frequently used thresholds are 6.8 nmol/L and 148 pmol/L for folate and vitamin B_{12}, respectively (14). According to these thresholds, we determined a deficiency of 17.8% for vitamin B_{12}, and 8.5% for folate in our study's population. In our study, although the prevalence of vitamin B_{12} deficiency varied depending on age and gender, the highest rate for both genders was found in the 71-80 age group. The prevalence of folate deficiency was similar in both genders, with the highest rate in the 18-30 age group. When we evaluated the entire population, it was found that men had a higher prevalence

Table 4. RIs for serum folate according to age subgroups

Groups	F/M*	Ages (Mean ± SD)	Folate, nmol/L (Mean ± SD)	RIs	
				Lower (90%CI)	Upper (90%CI)
18-30	1.721/597	23.7±3.5	12.3±5.8	4.9 (4.4-5.4)	27.7 (24.9-30.5)
31-40	1.566/459	35.7±2.8	13.2±6.1	5.5 (4.7-5.7)	29.9 (26.9-32.9)
41-50	2.089/620	45.6±2.9	14.2±6.4	5.5 (5.0-6.1)	30.9 (27.8-34.0)
51-60	2.149/813	55.4±2.8	14.8±6.7	5.6 (5.0-6.2)	30.9 (27.8-34.0)
61-70	1.671/798	65.1±2.8	14.5±6.4	4.9 (4.4-5.4)	31.9 (28.7-35.1)
71-80	1.058/642	75.0±2.7	13.1±6.6	4.9 (4.4-5.4)	31.3 (28.2-34.4)
All	10.254/3.929	49.7±16.6	13.9±6.6	5.2 (4.6-5.7)	30.9 (27.8-33.9)

F/M (female/male): Number of female/male patients, there is a significant difference among the all age groups (p<0.001), N: Number of patients, SD: Standard deviation, CI: Confidence internal

of both vitamin B_{12} deficiency and folate deficiency compared to women.

Clinical biochemistry laboratories share a massive database. We selected the indirect method to determine the RIs of vitamin B_{12} and folate because of the volume of data for test results. The advantages of the indirect method include the fact that it is less expensive and less time-consuming than the direct method. In addition, it allows for the detailed evaluation of population subgroups and the division of these groups, if necessary. Furthermore, studies have recommended that the indirect method can be as accurate as the direct method when the truth indirect is applied (3,6,11). Namely, exclusion criteria, preanalytical variables, and the analytical performance of the laboratory are very important in the application of the indirect method. In our study, we excluded people with diseases that would affect the level of the vitamins we studied, including people with chronic diseases such as malignancy, chronic kidney failure, chronic heart failure, chronic blood diseases, thyroid diseases, diabetes mellitus and patients with vitamin deficiency. We also excluded hemolysis samples that could impact preanalytical test results. In our study, 35.9% of patient records were deleted when all exclusion criteria were applied. The participants were selected from a pool of patients who applied for general examinations and who had diseases which were not expected to affect vitamin B_{12} and folate metabolism. The patient density was mostly higher in polyclinics, such as internal medicine, physical therapy and rehabilitation, dermatology, ophthalmology, and orthopedics.

Our study has several limitations. The first limitation was that we could not evaluate hematological parameters according to the laboratory data we used to determine the reference range. Measuring B_{12} and folate concentrations is a key for detecting vitamin deficiencies. However, homocysteine, methylmalonic acid tests, and other hematological tests that investigate varying levels of deficiency in these vitamins facilitate the diagnosis of deficiency and the evaluation of the status of these vitamins. Therefore, it is necessary to evaluate the hematological parameters to demonstrate their effectiveness regarding clinical decisions related to RIs. The second limitation of our study is that diseases that affect the vitamin levels of our patients (according to the diagnoses found in the hospital information system) or other additional factors are excluded. However, it is difficult to detect preanalytical factors that may affect these test results. In this regard, the results can be confirmed by a direct reference range determination method of a healthy population. However, in this case, this is always taken into consideration. Laboratory data contain serious medical information. Thus, the indirect method is more advantageous, financially efficient, and easier to follow than the direct method, especially when considering the extraction of substantial laboratory data with determined exclusion criteria in the process of determining a population's specific reference range.

Conclusion

The use of true RIs is an essential responsibility for laboratorians. There are different geographical regions, different climates, different eating habits, and different socioeconomic conditions in our country. In this respect, it is also important to determine RIs according to region. In our study, we determined RIs of our region for vitamin B_{12} and folate using the indirect method and a vast quantity of laboratory data. Furthermore, our study is a key for the comparison of the RIs recommended by the manufacturing company, and also for the determination of vitamin B_{12} and folate concentrations in the population at hand.

Table 5. The prevalence of vitamin B_{12} and for folate deficiency in our study population

Groups	Vitamin B_{12}				Folate			
	F/M*	Female (%)	Male (%)	All (%)	F/M*	Female (%)	Male (%)	All (%)
18-30	406/404	15.9	16.2	16.0	188/111	10.9	18.6	7.5
31-40	457/1.067	18.7	13.0	17.3	121/52	7.7	11.3	9.2
41-50	494/178	15.7	17.5	16.1	123/64	5.9	10.3	6.3
51-60	473/232	15.6	17.7	16.2	94/76	4.4	9.3	6.0
61-70	491/226	21.9	18.0	20.5	92/85	5.5	10.7	7.9
71-80	293/230	22.1	26.9	24.0	93/102	8.8	15.9	11.5
All	2.614/1.112	17.7	18.2	17.8	711/490	6.9	12.5	8.5

*: Number of vitamin B_{12} and folate deficiency in female/male, F/M: Female/male

Ethics

Ethics Committee Approval: This study was approved by the Clinical Research Ethical Committee of Medicine Faculty of Balıkesir University (date: 21.09.2020, number: 2020/168).

Informed Consent: The results were obtained retrospectively from the laboratory information system of Balıkesir State Hospital between the years of 2017 and 2019. The data used were approved by the hospital administration.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: M.A., M.F.A., Design: M.A., M.F.A., Data Collection or Processing: M.A., M.F.A., Analysis or Interpretation: M.A., M.F.A., Literature Search: M.A., M.F.A., Writing: M.A., M.F.A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

References

1. Schwettmann L, Berbu S. Reference Interval and Status for Serum Folate and Serum Vitamin B12 in a Norwegian Population. Clin Lab 2015;61(8):1095-1100.
2. CLSI. Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline-Third Edition. CLSI Document C28-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
3. Jones GR, Haeckel R, Loh TP, Sikaris K, Streichert T, Katayev A, et al. Indirect methods for reference interval determination - review and recommendations. Clin Chem Lab Med 2018;57(1):20-29.
4. Laleli Y, Akbay A. Tıbbi laboratuvarlarda standartdizasyon ve kalite yöntemi kitabı. In Laboratuvar verilerinin istatistiksel değerlendirmesi, Taga Y, Aslan D, Güner G, Kutay FZ, (editors). 1st ed., Ankara: Mart Yayıncılık, 2000:124-137.
5. Institute of Medicine. Dietary Reference Intakes: Thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. Washington, DC: National Academies Press, 1998.
6. Bakan E, Polat H, Ozarda Y, Ozturk N, Baygutalp NK, Umudum FZ, et al. A reference interval study for common biochemical analytes in eastern turkey: a comparison of a reference population with laboratory data mining. Biochem Med (Zagreb) 2016;26(2):210-223.
7. Köseoğlu M, Işleten F, Dursun S, Çuhadar S. Determination of reference intervals of healthy adults aged between 20-50 years in Izmir. Turk J Biochem 2010;35(3):215-224.
8. Önder Y, Tezcan S, Kadılar ÖK, Köroğlu Dağdelen L, Madenci Çakır Ö, Yücel N, et al. Determination of vitamin B12 and folic acid Reference intervals at Kartal district. J Turk Clin Biochem 2014;12(3):131-136.
9. İlçöl YÖ, Aslan D. Determination of reference intervals of the healthy subjects in Bursa. Turk J Biochem 2004;29:183-192.
10. Oncel M, Sahin, E, Kiyici A, Adam B. Use of laboratory information system data for indirect estimation of reference interval for vitamin B12. J Lab Med 2015;39(6):437-441.
11. Avcı E, Aslan D. Determination of indirect reference intervals of vitamin B12 for outpatients admitted to primary healthcare centers. J Lab Med 2020;44(1):11-16.
12. Tanyalcin T, Aslan D, Kurtulmus Y, Gökalp N, Kumanlioğlu K. Reference intervals of serum folate and vitamin B12 developed from data of healthy subjects. Accred Qual Assur 2000;5(9):383-387.
13. McLean E, de Benoist B, Allen LH. Review of the magnitude of folate and vitamin B12 deficiencies worldwide. Food Nutr Bull 2008;29(Suppl 2):S38-S51.
14. Wong CW. Vitamin B12 deficiency in the elderly: is it worth screening? Hong Kong Med J 2015;21(2):155-164.