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Use of laboratory information system data for indirect estimation of reference interval for vitamin B12

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Abstract

Background: In common, clinical laboratories use reference intervals recommended by the manufacturers. Various factors affect laboratory tests such as age, sex, diet and genetics. So, it is recommended for each laboratory to determine its own reference ranges for each test used. We aimed to establish our reference interval for vitamin B12.
Methods: The data archive of laboratory information system was searched for a 1-year period between January and December, 2013. Among 2526 subjects searched for vitamin B12, 2368 remained (1–70 years old, 512 male and 1856 female) when we excluded the outliers for estimation of reference range for vitamin B12 with nonparametric method according to National Committee for Clinical Laboratory Standards (NCCLS) C28-A3 guidelines. Serum levels of vitamin B12 were determined with electrochemiluminescent technique.
Results: New reference interval for vitamin B12 derived from our results was 101–702 pg/mL, and was not affected by gender.
Conclusions: New reference interval was different from the one recommended by the manufacturer. We suggest that established reference interval reflects our population better than the values recommended by the manufacturer.

Keywords: data mining; indirect method; reference interval; vitamin B12.

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Introduction

The objective data provided by the clinical laboratories is important in terms of making critical medical decisions such as diagnosing a patient, treatment selection and monitoring the treatment. Incorrect reference intervals used by the laboratories will cause a series of problems such as misdiagnosis, unsuitable treatment applications and unnecessary test repeats [1]. Moreover, broad reference ranges may cause subclinical statements to be masked; whereas narrow reference ranges may cause misdiagnosis. Therefore, the correct reference intervals determined by clinical laboratories will reduce the risk of false negative and false positive results in the interpretation of test results [2].

Today, generally each laboratory uses the reference interval, which is recommended by the manufacturer. However, these reference intervals are not always appeal to the characteristics of that population [3]. Therefore, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and the Clinical and Laboratory Standards Institute (CLSI) suggest each laboratory to determine its own reference intervals [2, 4].

In clinical laboratories for determination of reference intervals, methods recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (nonparametric) and IFCC (parametric) were commonly used [5–7]. Although nonparametric method is appropriate for non Gaussian distribution of the values, NCCLS recommends this method because of less complicated statistical calculations either in parametrically distributed data. Besides the indirect method for estimation of clinical reference intervals from total hospital patient data was highly recommended because this method is cost effective and less time consuming. It is very difficult for a laboratory to determine reference intervals for all analytes by using direct methods which need sampling from at least 120 healthy individuals, application of a standard questionnaire and calculation of reference intervals. However, indirect methods have some advantages: larger sample size due to use of total hospital

patient data, no questionnaire, less complicated statistical calculations, and reflecting the investigated population better. Therefore, establishment of reference intervals from the archive of the laboratory information system is commonly used [8–12].

Vitamin B12, which is a water soluble vitamin has some regulatory functions in the hematopoetic and nervous systems [13]. The deficiency of vitamin B12 is observed with a ratio of 15%–40% in adults and elderly patients, however, the rate of the diagnosed cases is much lower because most of them do not present clinical symptoms or are misdiagnosed [14].

We aimed to determine the reference interval for vitamin B12 specific to our region by using the archive of laboratory information system and evaluate whether it is consistent with the reference interval recommended by the manufacturer.

Materials and methods

Reference group

We searched the archive of laboratory information systems for establishment of reference interval for vitamin B12 and achieved a total number of 2526 test results for vitamin B12 registered to Mevlana University Hospital Laboratory Information System (Meddata, Ankara, Turkey) between January 2013 and December 2013. In the case of multiple test results for the same patient, only the first test result was included in the study. Pregnant women, patients with chronic renal failure or from the intensive care unit were excluded from the study. Patients with a diagnosis of megaloblastic anemia, pernicious anemia and peripheral neuropathy, due to cobalamin deficiency were also excluded.

Analysis

Vitamin B12 measurements were performed by electrochemiluminescence method in the Elecsys 2010 auto analyzer (Roche Diagnostics, Ltd., Rotkreuz, Switzerland) using original Cobas vitamin B12 reagents (Roche Diagnostics, Ltd., Rotkreuz, Switzerland). Recommended reference interval by the manufacturer is 211–946 pg/mL.

Determination of reference intervals

The distribution of vitamin B12 in our population was tested with the Shapiro-Wilk test. Although it presents normal distribution (p=0.114), we used the nonparametric method (NCCLS C28-A) for calculating the reference interval as it was suggested previously [8–12]:

1. The histogram of data was plotted and examined.
2. The possibility of the existence of sub-groups was tested with the Student's t test and one way ANOVA.
3. Extreme values were excluded (according to the D/R rule).
4. Testing the gender specific differences was performed with standard deviation tests: Z value and critical Z value was calculated. Because $Z_{calculated} < Z_{critical}$ there was no significant difference in reference interval for males and females.
5. Patients were divided into seven groups starting from first decade to the seventh decade and reference interval of vitamin B12 was determined for these groups.
6. Lower and upper limits were calculated on the basis of those considered individually if different, or together if there is a similarity rate of 95%.

$$\text{Lower limit} = 0.025 \times (n+1)$$
$$\text{Upper limit} = 0.075 \times (n+1)$$

n=number of the data

7. The confidence interval (95%) of upper and lower limits was also calculated [15].

Excluding extreme values [5, 6, 15]: The rule of D/R 1:3 was applied: D=the end value–the value nearby, R=range value between all data, if D/R>0.33 then the value was not considered in the calculation.

Statistical analysis

SPSS version 15.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Distribution characteristics of the numeric values were tested with Shapiro-Wilk test. The gender difference was examined by the Student's t test, while difference between age groups based on decades was determined by one-way ANOVA test; $p<0.05$ was considered statistically significant.

Results

Analytical quality control and analytical performance studies

Quality control and analytical performance studies were performed for vitamin B12 test used in our laboratory. The Vitamin B12 CalSet II calibrator (Roche Diagnostics, Ltd., Rotkreuz, Switzerland) was used for calibration and Precicontrol Varia 1 and Precicontrol Varia 2 (Roche Diagnostics, Ltd., Rotkreuz, Switzerland) control materials were used for internal quality control, respectively. Two thousand and thirteen external quality assessments of vitamin B12 were performed by using KBUDEK External Quality Control Program.

Intra-assay and inter-assay CV and recovery analytical performance assessments were performed using serum pools with low and high vitamin B12 levels. Intra-assay CV was determined as 4.08% for low (133±5.4 pg/mL), and 3.27% for higher (609±20 pg/mL) concentrations; inter-assay CV was determined as 9.06% for low and as

4.10% for high level serum pools. Recovery was determined as 90.9% for low and 142% for high levels. The detection range of vitamin B12 determined by the manufacturer was 30–2000 pg/mL, and the limit of quantification was 34.69 pg/mL.

Calculation of reference intervals

After excluding the extreme values, the reference population consisted of the remaining 2368 individuals. The mean age and age range of the reference population was 39 years (1–70 years). Five hundred and twelve of 2368 individuals were female and the remaining 1856 individuals were male. The reference interval established for the whole population was 101–702 pg/mL. The reference interval recommended by the manufacturer was 211–946 pg/mL. Reference intervals for females and males were 102–700 pg/mL and for female 101–702 pg/mL, respectively. There was no significant difference when age specific reference intervals were calculated for vitamin B12 levels in our population (p=0.620). We did not find any significant difference for different age groups (p=0.440), so we suggest using of the same reference interval for all ages (Table 1).

Discussion

Reference intervals were determined with parametric and non-parametric methods in accordance with recommendations of the NCCLS and IFCC. IFCC recommends both parametric and non-parametric methods, while the NCCLS recommends non-parametric methods to calculate the reference ranges [16].

The most ideal reference interval is the individual reference interval obtained from previous data of the subject. However, this is not always possible. Therefore, community-based reference intervals are determined frequently. In the direct sampling method; healthy, non-obese, non-pregnant and non-smoking people with no alcohol consumption and no history of drug use should be selected. However, when these criteria are applied, it is very difficult to create a reference interval reflecting the general population [11].

Three types of reference range are defined: tolerance range, the expected range and 95% inter-percentile range. The inter-percentile range method, which is easily determined and the most common one, is accepted by IFCC. In this method, the distribution between 2.5% and 97.5% determines the reference range [17].

Due to the high costs and difficulty of determining the appropriate population in the direct sampling method, which is also time consuming, each laboratory may create its own reference interval from the archive of the laboratory information system [10, 18–22].

As most of the tests are affected by various pre-analytical factors such as environment, genetics, diet, gender, age, etc., use of reference intervals recommended by the manufacturer cause problems in diagnosis. Misdiagnosis, unnecessary test repeats, implementation of unsuitable treatment procedures cause both patients and physicians to waste their time [16, 20].

In our study, the reference interval established for vitamin B12 was not consistent with the one which was recommended by the manufacturer. Both the lower and upper limits we determined (101–702 pg/mL) were lower than the ones recommended (191–663 pg/mL). Intra- and inter-assay CV values of our assay were below the desirable within-subject biological variation (CV_I=15%) [23]. When we evaluate the subjects in our population for vitamin B12 deficiency either with the medical decision limit for this test (170 pg/mL) or a cut-off value for diagnosis of vitamin B12 deficiency (200 pg/mL), the incidence of vitamin B12 deficiency is 4%–5%. Our lower reference limit is lower than both the medical decision limits (170 pg/mL and 250 pg/mL) [23] and the clinical cut off value (200 pg/mL). For subjects with a vitamin B12 value between 101 and 170 pg/mL, they should be evaluated for clinical symptoms and monitored carefully before deciding on replacement of vitamin B12.

In two similar reference interval studies conducted in our country, researchers determined new reference intervals which were different from the reference intervals recommended by the manufacturer for vitamin B12. Our reference interval was different from the values reported in these two studies. According to Demirin et al., the reference range of vitamin B12 was determined as 158.0–563.9 pg/mL for the Black Sea region [24]; while Özarda et al. have

Table 1: Reference intervals of vitamin B12 (pg/mL) for different age groups.

Number of probands	Reference interval	95% CI LB[a]	95% CI UB[b]
0–10 (n=31)	230–679	228–232	677–691
11–20 (n=245)	122–698	120–124	696–700
21–30 (n=580)	118–700	116–120	698–702
31–40 (n=579)	107–698	105–109	696–700
41–50 (n=404)	102–689	100–104	687–701
51–60 (n=269)	101–700	99–103	698–702
61–70 (n=260)	100–702	98–102	700–704

[a]95% CI LB: The lower bound of 95% confidence interval. [b]95% CI UB: The upper bound of 95% confidence interval.

proposed a reference interval as 214–1544 pg/mL for males and 319–1996 pg/mL for females in Bursa [16]. In the study by Oncel et al., conducted on a total of 889 elementary and high school students within 12–22 years of age living in the city center of Diyarbakır, the gender distribution was as follows; 294 females (33.1%) and 595 males (66.9%), respectively. The average vitamin B12 level was determined as 331.51±144.05 pg/mL (325.60±138.91 pg/mL in males, and 343.48±153.48 pg/mL in females, respectively) [25]. In a parametrically designed reference interval study for vitamin B12 performed in Konya by Akin et al., 1109 samples were analyzed for vitamin B12 levels; 54 from cord-blood and 1055 from healthy subjects aged 0–24 years. The reference interval for vitamin B12 in the confidence interval of 2.5–97.5 percentile (P2.5–P97.5) was 127–606 pg/mL for females, 127–576 pg/mL males, and 127–590 pg/mL for the entire population [26]. The reference interval determined in our study is close to the values determined by Demirin et al. [24] and Akin et al. [26].

In our study, there was no significant difference for the reference interval of vitamin B12 in terms of gender and age. In most of the studies conducted in our country, the authors did not suggest gender specific reference intervals. On the other hand, Ozarda et al. have proposed different reference interval for males and females. According to their report, both upper and lower limits determined for females are higher than the limits determined for males.

Considering the studies conducted in other countries; Papandreou et al. have divided a total of 524 (275 male, 249 female) healthy Greek children into three groups: 6–9 (group 1), 10–12 (group 2), 13–15 (group 3) based on their ages and they have found reference intervals for vitamin B12 as 1048 (117–2000), 805 (296–2000), 700 (214–2000) pg/mL, respectively. In addition, they did not find any significant difference for reference intervals of vitamin B12 in terms of gender [27].

Akanji et al. have reported the reference interval of a total of 774 (316 male, 458 female) healthy Arabs (between 10 and 19 years of age) as 354.3 pg/mL (343.0–365.7) [28], while Galukande et al. have determined the reference interval of vitamin B12 as 117–1158 pg/mL for young people living in Uganda [13].

The reference interval values recommended by different authors for vitamin B12 vary from each other. Even in different geographic areas of our country, these values are different. Therefore, it is important for each laboratory to establish its own reference interval for each analyte.

We can conclude that laboratory-specific reference interval can be established from the data archive of laboratory information systems. This is easy and inexpensive as well. Finally, using a more precise and true reference interval for vitamin B12 would be more suitable for diagnosis or treatment instead of using the values suggested by the manufacturer.

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References

1. Carraro P, Plebani M. Errors in a stat laboratory: types and frequencies 10 years later. Clin Chem 2007;53:1338–42.
2. Bailey D, Colantonio D, Kyriakopoulou L, Cohen AH, Chan MK, Armbruster D, et al. Marked biological variance in endocrine and biochemical markers in childhood: establishment of pediatric reference intervals using healthy community children from the CALIPER cohort. Clin Chem 2013;59:1393–405.
3. Tezcan S, Ercan Ş, Çakır Madenci Ö, Yücel N, Orçun A. Determination of reference intervals for CA15-3, CA 19-9, CA 125 and CEA. Turk J Clin Biochem 2013;11:15–20. [Turkish].
4. Erdoğan S, Erden G, Yıldırımkaya M. Determination of rerefence interval for Elecyss 2010 insulin test in Turkish people between 18–60 years. Turk Bull Hygiene Exp Biol 2009;66:66–72. [Turkish].
5. NCCLS C28-A. How to define and determine reference intervals in the clinical laboratory; approved guideline, NCCLS, 1995;17, No 18.
6. CLSI C28-A3. Defining, establishing, and verifying reference intervals in the Clinical Laboratory; Approved Guideline, 3rd ed. CLSI document C28-A3. Wayne, PA: Clinical and Laboratory Standards Institute, 2008.
7. Solberg HE. Approved recommendation on the theory of reference values. Part 5. Statistical treatment of collected reference values. Determination of reference limits. J Clin Chem Clin Biochem 1987;25:645–56.
8. Masferrer MF, Arderiu XF, Ane RP. Indirect reference limits estimated from patients results by three mathematical procedures. Clin Chim Acta 1999;279:97–105.
9. Baadenhuijsen H, Smit JC. Indirect estimation of clinical chemical reference intervals from total hospital patient data: application of a modified Bhattacharya procedure. J Clin Chem Clin Biochem 1985;23:829–39.
10. Kairisto V, Hänninen KP, Leino A, Pulkki K, Peltola O, Näntö V, et al. Generation of reference values for cardiac enzymes from hospital admission laboratory data. Eur J Clin Chem Clin Biochem 1994;32:789–96.
11. Kouri T, Kairisto V, Virtanen A, Uusipaikka E, Rajamaki A, Finneman H, et al. Reference intervals developed from data for hospitalized patients: computerized method based on

combination of laboratory and diagnostic data. Clin Chem 1994;40:2209–15.
12. Solberg HE. Using a hospitalized population to establish reference intervals: pros and cons. Clin Chem 1994;40:2205–6.
13. Galukande M, Jombwe J, Fualal J, Baingana R, Gakwaya A. Reference values for Serum levels of Folic acid and Vitamin B_{12} in a young adult Ugandan population. African Health Sciences 2011;11:240–3.
14. Andres E, Serraj K. Optimal management of pernicious anemia. J Blood Med 2012;3:97–103.
15. Sasse EA. Determination of reference intervals in the clinical laboratory using the proposed guideline National Committee for clinical laboratory standarts C28-P. Arch Pathol Lab Med 1992;116:710–3.
16. Özarda İlçöl Y, Aslan D. Determination of reference intervals for blood chemistry profiles in healthy individuals in Bursa. Turk J Biochem 2004;29:183–92. [Turkish].
17. Taga Y, Aslan D, Güner G, Kutay FZ. Standardization and Quality Management in Medical Laboratories. A production of Turkish Biochemical Society 2008;131. [Turkish].
18. Bhatia P, Kulkarni JD, Pai SA. Vitamin B12 deficiency in India: mean corpuscular volume is an unreliable screening parameter. Natl Med J India 2012;25:336–8.
19. Baadenhuijsen H, Smit JC. Indirect estimation of clinical chemical reference intervals from total hospital patient data: application of a modified Bhattacharya procedure. J Clin Chem Clin Biochem 1985;23:829–39.
20. Inal TC, Serteser M, Coşkun A, Ozpinar A, Unsal I. Indirect reference intervals estimated from hospitalized population for thyrotropin and free thyroxine. Croat Med J 2010;51:124–30.
21. Krøll J, Saxtrup O. On the use of patient data for the definition of reference intervals in clinical chemistry. Scand J Clin Lab Invest 1998;58:469–73.
22. Ilcol YO, Aslan D. Use of total patient data for indirect estimation of reference intervals for 40 clinical chemical analytes in Turkey. Clin Chem Lab Med 2006;44:867–76.
23. Westgard QC. Medical Decision Levels. Tools, Technologies and Training for Healthcare Laboratories. Available at: http://www.westgard.com/decision.htm. Accessed 17 Jan 2014.
24. Demirin H, Memişoğullari R, Uçgun T, Yildirim HA, Celer A, Bulur Ş, et al. Are reference intervals suitable for anemia parameters such as iron, ferritin, vitamin B12 and folate in Turkish adults living in West Black Sea Region? Turk J Biochem 2012;37:356–61. [Turkish].
25. Öncel K, Özbek MN, Onur H, Söker M, Ceylan A. Vitamin B12 and folic acid in children and adolescents in Diyarbakir. Dicle Med J 2006;33:163–9. [Turkish].
26. Akin F, Yavuz H, Bodur S, Kiyici A. Vitamin B_{12} levels of subjects aged 0–24 year(s) in Konya, Turkey. J Health Popul Nutr 2014;32:615–22.
27. Papandreou D, Mavromichalis I, Makedou A, Rousso I, Arvanitidou M. Total serum homocysteine, folate and vitamin B12 in a Greek school age population. Clin Nutr 2006;25:797–802.
28. Akanji AO, Thalib L, Al-Isa AN. Folate, Vitamin B_{12} and total homocysteine levels in Arab adolescent subjects: reference ranges and potential determinants. Nutr Metabol Cardiovasc Dis 2012;22:900–6.