Original Article

The reference value of serum vitamin B12 among An-Najah National University

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Abstract

Background: Serum vitamin B12 plays a crucial role in cellular growth, DNA synthesis, and maintaining a healthy nervous system. Its deficiency can lead to various health issues, including cardiovascular problems. The reference range for vitamin B12 varies between populations due to cultural, genetic, and dietary differences. **Methods:** A cross-sectional study was conducted on 127 healthy An-Najah National University students aged between 18 and 25. Blood samples were collected, and a questionnaire assessed factors affecting vitamin B12 levels. Serum vitamin B12 levels were measured using ACCUBIND ELIZA microwells. **Results:** The study found a reference range for serum vitamin B12 of 136–640 pg/mL (5^{th} to 95^{th} percentile) among the studied population, which differed from the manufacturer's claimed range of 200–835 pg/mL. Gender did not significantly affect the reference intervals. **Conclusion:** The study highlights the importance of establishing local reference values for laboratory tests like vitamin B12 due to variations in demographics. Further research in different Palestinian regions and age groups with larger sample sizes is recommended to refine these reference values.

Keywords: ACCUBIND ELIZA microwells, An-Najah National University student, cross-sectional study, reference range, serum vitamin B12

Introduction

Vitamin B12, a type of vitamin that dissolves in water, is essential, for cell growth and development as it helps with creating DNA-producing blood cells and keeping the nervous system in good condition.[1] When vitamin B12 levels are low, homocysteine (Hcy) is converted to methionine. Elevated homocysteine levels, due to a lack of vitamin B12, can contribute to heart disease and the hardening of arteries known as atherosclerosis.[1] Vitamin B12, although produced in the intestine by microflora, is advised to be acquired from food because its synthesis occurs away, from the absorption site.[2]

Red and white meat, in addition to dairy products, have lots of vitamin B12. However, people who do not consume animal

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products are facing a greater chance of not having enough B12 in their bodies.[3–5] The prevalence and scope of vitamin B12 deficiency remain uncertain due to variations in lifestyle and genetic diversity across different regions.[6] Inadequate levels of B12 can lead to megaloblastic anemia, nerve damage, cognitive decline, and cardiovascular issues attributed to elevated homocysteine levels in the bloodstream.[5,7]

Numerous factors, including age, gender, weight status, and geographical location, can influence vitamin B12 levels.[8,9] For instance, a study conducted in Jordan revealed a higher prevalence of low vitamin B12 levels among overweight boys.[10] Similarly, research conducted in Palestine identified a correlation between *Helicobacter pylori* infection and reduced levels of vitamin B12 in adults, particularly indicating a potential link to anemia and gastritis.[11]

Research indicates a significant relationship between smoking and metformin usage and alterations in vitamin B12 levels. For instance, a study conducted in Udaipur revealed that constant cigarette smokers exhibited lower levels of vitamin B12 compared to nonsmokers.[12] Similarly, in Saudi Arabia, a separate investigation demonstrated that metformin utilization could lead to a reduction in vitamin B12 levels by 57 pmol/L, potentially resulting in deficiencies among certain type 2 diabetes patients.[13]

Vitamin B12 levels exhibit significant variation across diverse demographic groups and regions. Relying on reference levels from a different population could potentially lead to erroneous conclusions.[14,15] Therefore, establishing country-specific reference ranges is crucial for accurate assessment.

This research significantly contributes to the understanding of factors influencing vitamin B12 levels and underscores the importance of utilizing country-specific reference ranges for accurate diagnosis and treatment. By elucidating the nuances of vitamin B12 regulation, it empowers healthcare professionals to effectively identify and address deficiencies, ensuring that patients receive appropriate care tailored to their individual needs.

Numerous studies have delved into the realm of vitamin B12, yet their scope is not without limitations. One notable gap lies in the insufficient exploration of how cultural backgrounds and genetic predispositions influence B12 levels across diverse demographics. Additionally, there remains a pressing need for further investigation into the efficacy of various treatments for addressing low B12 levels. Expanding on these fronts could provide invaluable insights into optimizing B12 management strategies.

In conclusion, this study delves into the factors influencing vitamin B12 levels and underscores the significance of tailored laboratory test values for each country. By retaining citation numbers, this research facilitates the understanding and verification of its findings, enabling healthcare professionals to accurately diagnose and manage low vitamin B12 levels. Consequently, patients can receive the precise care necessary to safeguard their health and well-being.

Methodology

This is a cross-sectional study conducted from 23rd of October to 13th November. A total of 127 healthy (27 of males) and (100 of females) subjects were screened aged between 18 and 25. The study was performed in the laboratories of medicine and health sciences department at An-Najah National University in Nablus, Palestine. Our study populations were healthy university students. A questionnaire was provided and blood samples were withdrawn under the approval of the IRB at NNU ethical approval number (31) sep. 2019.

Subjects were recruited through announcements on social media and the Facebook page of the university of students.

Blood samples were obtained from 127 healthy individuals after filling a questionnaire of 20 items about factors that can affect the concentration of serum vitamin B12, the questionnaire was abstracted from a study in Konya, Turkey,[16] that was divided into three sections: the first section of the questionnaire was about the age, sex, weight, height, BMI, and monthly income of parents.

The second section included questions about diseases, drugs, B12 supplements, smoking, inflammation, and operations.

- The last section was about the nutritional habits of the study group, red meat and seafood consumption, and whether they follow specific diets. As red meat is the most important source of vitamin B12, the study included subjects who consumed red meat at least twice per week and excluded those who consumed less. It is worth noting that since the questionnaire was created solely by the authors, it cannot be regarded as a universal tool for measuring validity.

Regarding the inclusion/exclusion criteria, university students apparently healthy aged between 18 and 25 were included in this study.

However, the study was designed to establish serum B12 levels, and then, obviously subjects who suffer from B12 deficiency and anemia are excluded.

Other exclusions are, diabetes, malabsorption, gastrointestinal disease or surgery, and parasitic infections.

As the most important source of vitamin B12 is red meat, we considered that vegetarians and individuals with nutritional deficiency or consuming less than twice per week of meat were been excluded either. Other exclusion criteria were smoking and pregnancy. Also, individuals on treatment for vitamin B12 (vitamin B12 deficiency) or on drugs known to interfere with

the level of vitamin B12, such as metformin, peptic ulcer drugs, and chloramphenicol, were asked not to participate.

Approval was obtained from "The Institutional Review Board" (IRB) at An-Najah National University in Palestine prior to the research conduction. All study participants were freely accepted to join the study. Informed consent was obtained from the patients. All data were collected and treated confidentially and available for the researchers only. Other ethical safeguards will be employed: a self-reported questionnaire will be used and participation will be voluntary. Completed questionnaires will be collected in special boxes in private rooms to ensure participant's privacy. All data collected will be confidential and available for the researchers only. The names of the participants were not included in the results as requested by the IRB committee to avoid stigmatization issues.

The study did not have a large sample size, due to the inability to afford B12 kits, also we were unable to study all factors that affect B12 concentrations as this requires many analyses.

1. Blood samples were collected by standard venipuncture into evacuated tubes without anticoagulant. The blood tubes were centrifuged to separate serum from the whole blood. The serum was stored at −80°C until analysis. Serum VB12 concentrations were determined by delayed competitive enzyme immunoassay (ACCUBIND ELIZA Microwells; Monobind INC).
2. Read the absorbance in each well at 450 nm (using a reference wavelength of 620–630 nm. The results should be read within fifteen (15) minutes adding the stop solution.

Data analysis was done using SPSS version 26; descriptive analyses and Spearman rank correlations methods were used.

Results

Of the 200 students who joined in the study, 127 samples were included and tested for vitamin B12, 100 of them were female (78.7%) and 27 were male (21.3%), 73 were excluded, due to either poor quality of the sample or they fell in the exclusion list. The values obtained from this study ranged from 136–640 pg/ml for vitamin B12 (5th to 95th percentile) [Figure 1]. And the average concentrations of vitamin B12 in the blood in men, Female, the total population was 363 pg/ml, 335 pg/ml, and 341 pg/ml, respectively. [Table 1]. There was no significance The difference in vitamin B12 concentrations between the six different ones.) After this paragraph(Because our data are not normally distributed, Spearman rank correlations method was used to give information about the degree of correlation as well as the direction of these confounders (BMI, Hb, and MCV); the degrees of correlation are shown in Table 2. Indicating a no correlation to a low degree of correlation, implying the tested for cofounders did not significantly influence the ranges we see in this study. As Table 2 shows that the body mass index, hemoglobin level, and MCV were not significant and they were not correlated with vitamin B12, moreover, they did not affect vitamin B12 level. Alcohol and cigarette smoking are not shown in the table, because the participants should be healthy, and to eliminate any factor that may affect the values of vitamin B12 level.)

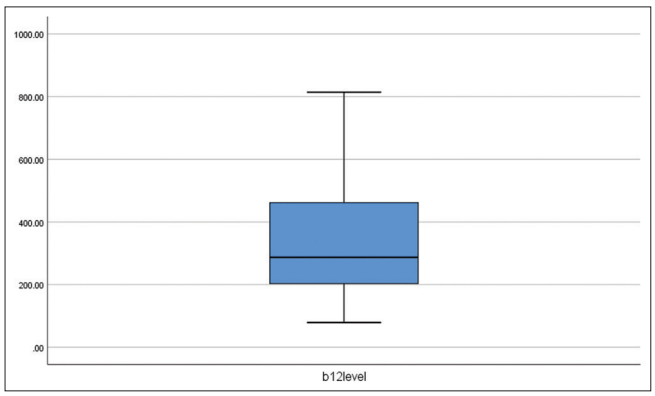

Figure 1: The mean of vitamin B12 level

Alcohol and cigarette smoking are not shown in the table, because the participants should be healthy, and to eliminate any factor that may affect the values of vitamin B12 level.

Figure 1 vividly demonstrates the average range of vitamin B12 levels within the gathered samples.

In this analysis, we have compiled statistics for four important parameters. After analyzing 127 samples, we found that B12 levels ranged widely from 78.79, to 814.13 with an average of around 341.0148 and a standard error of the SEM of 15.8. Hemoglobin (Hb) levels in 117 samples showed a range of 10.30 to 16.40 with a value of about 12.9444 and a SEM of 0.1304. Body mass index (BMI) based on data from 111 samples varied between values of 16.40 and 32.80 with a BMI of around 22.3342 and a SEM of about 0.3634. Additionally, mean corpuscular volume (MCV) was measured in samples from the group of individuals ranging from values as 69 up to as high as 90.70 with the average MCV being approximately 81.9863 and a SEM

Table 1: Different parameters descriptive analysis

	n	Range	Minimum	Maximum	Mean	SEM
B12 level	127	735.34	78.79	814.13	341.0148	15.8
Hb level	117	6.10	10.30	16.40	12.9444	0.1304
BMI	111	16.40	16.40	32.80	22.3342	0.3634
MCV	117	21.70	69.00	90.70	81.9863	0.3549

Table 2: Spearman correlations of vitamin B12 with BMI, Hb, MCV

Element	B12
Hb	−0.018
Sig.(2-tailed)	0.845
BMI	−0.048
Sig.(2-tailed)	0.620
MCV	0.145
Sig.(2-tailed)	0.118

calculated at around 0.3549. These statistics provide us with an overview of the dataset offering insights into the central tendencies and variability observed in these parameters that are crucial, for our research interpretation. Data are illustrated in Table 1.

Figure 2 illustrates the gender differences observed in vitamin B12 levels, hemoglobin levels, BMI, and mean corpuscular volume. On average, males tend to have slightly higher vitamin B12 levels compared to females. Additionally, males exhibit higher hemoglobin levels compared to females. Moreover, males tend to have higher body mass index (BMI) on average compared to females. Furthermore, there is a slight difference in mean corpuscular volume (MCV) between genders, with males having a slightly higher average MCV compared to females.

Discussion

The reference range of laboratory test is important to give an indication if the patient is healthy or exposed to some pathological condition. However, there is not a universally agreed-upon threshold to definitively identify B12 deficiency. In fact, deficiency can sometimes manifest even when B12 levels fall within the accepted normal range.[17] So, each laboratory should establish its own reference values because it depends according to different demographic factors and also differs from country to country. However, this recommendation looks to have had limited influence in most clinical laboratories. Therefore, most laboratories depend on their result on the reference value provided by the manufacture using the kit, regardless of where and how these values are calculated, but this may give false positive results. The cause of this is the fact that the calculation of an adequate reference interval is a highly demanding and difficult activity.[15] The Clinical Laboratory Standard Institute (CLSI) and the International Federation of Clinical Chemistry (IFCC) recommend the inclusion of at least 120 individuals;[18] in the calculation of a reference interval, this recommendation is fulfilled in our sample (n = 127).

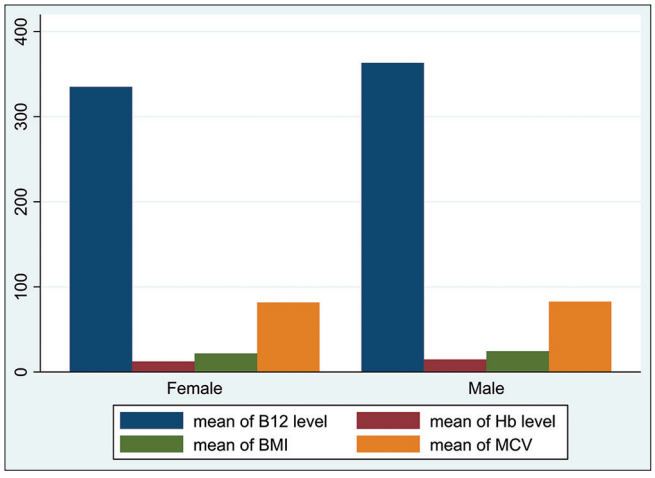

Figure 2: Means vitamin B12 level, BMI, MCV, and Hb level according to gender

In our study, good health students, not receiving any vitamin or drug, and those consuming an adequate amount of meat were included for measuring the normal range of vitamin B12 which was in our study 136–640 pg/ml (5th to 95th percentile). The reference range from the laboratory kit was 200–835 pg/ml. Another research endeavor focusing on ethnic diversity demonstrated that when employing standardized reference intervals (RIs), only 2.2% of Black individuals were categorized as "deficient," contrasting with 6% of Asian, 5.5% of White, and 4.2% of Mixed race individuals.[19] The examined cohort indicated that by adopting the RIs values established in this particular study, the occurrence of vitamin B12 deficiency would have been 5% among Black patients and 4.6% (RIs Black (pmol/L) 166–805) among Asian/White patients within the cohort (RIs Asian/White (pmol/L) 134–511) for the age group above 13 years old.[20]

For vitamin B12, we obtained a five percentile considerably lower than the one claimed by the manufacturer (200 vs. 136 pg/mL) and also at a 95 percentile (835 vs. 640 pg/mL). The findings of another study conducted in Turkey unequivocally indicate variations in the lower and/or upper boundaries of reference intervals (RIs) obtained from direct and indirect methodologies across certain analytes, including GGT, ALT, TG, TC, HDL-C, LDL-C, ALB, Fe, UA, FT3, FT4, and vitamin B12. Notably, ALB and UA exhibit a broader RI when assessed via the indirect approach compared to the direct method. Conversely, vitamin B12 demonstrates analogous outcomes, with higher upper limits observed in the direct method.[21] This fact reinforces the need for each clinical laboratory to build its own reference intervals.

However, we applied the Spearman rank correlations method to our data to find sex-related differences between serum vitamin levels. Sex differences are not so clear cut in previously published studies,[20] and accordingly, we found no relation between reference intervals and sex.

Strengths

The strengths of this study lie in its methodology and adherence to standards. The researchers collected data from a group of 127 individuals aged between 18 and 25, which provided a comprehensive understanding of vitamin B12 reference values among university students in Palestine. They used questionnaires and blood serum samples to ensure data collection.

Limitations

However, there are limitations that should be taken into account. The small sample size was due to cost constraints related to vitamin B12 kits, which could potentially affect the generalizability of the findings. The exclusion criteria, such as excluding individuals with health conditions or lifestyles (smokers and pregnant individuals), may have limited the representation of certain subgroups in the study. Additionally, the research did not explore all factors that can influence vitamin B12 concentrations and there were differences between the reference values obtained

in this study and those provided by manufacturers. Lastly, since this study focused on An-Najah National University student in Nablus it raises questions about how applicable these findings are to the Palestinian population, given that reference values can vary based on regional and demographic factors.

Recommendations

In the light of the results shown in the study, we recommended that:

1. To expand the investigations to different regions of Palestine to determine the normal level of vitamin B12 among Palestinians.
2. To carry the study on different age groups.
3. To carry the study on a larger sample size than our sample.

Conclusion

The normal reference level obtained for vitamin B12 at the 5–95 percentile range was 136–640 pg/mL for the healthy, not vitamin-supplemented studied population, this interval differed from those claimed by the manufacturer. Vitamin B12 levels vary from country to country; comparisons between countries may not be valid, and normal levels for each population should be obtained.

Ethics approval and consent to participate

i. All experiments and tests were carried out according to relevant guidelines and regulations.
ii. The study protocol was approved by An-Najah National University IRB committee ethical approval number (31) sep. 2019.
iii. Written informed consent was obtained from patients prior to the interviews. We explained that the collected data will be used only for clinical research and that their confidentiality will be preserved.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

All authors contributed considerably to this work. Lubna Kharraz, Mohammad Alnees, Mahdi Awwad, and Duha Najajra were responsible for designing the study and drafting the manuscript. Mohammad Alnees and Lubna Kharraz analyzed the data. Aseel Shabaro, Rola Hazzam, Asma Saleem, Shatha Qaffaf, Dalia Hmiedan, Sima Abuhayyat, Reem Shekha, Majd Bawwab, Abdalaziz Darwish, Duha Najajra, Nizar Abu Hamdeh, and Mahdi Awwad were responsible for the data collection and interpretation. All authors reviewed, edited, and approved the final version of the manuscript.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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