Progress in Nutrition 2021; Vol. 23, N. 4: e2021166 DOI 10.23751/pn.v23i4.11009

Original article

Vitamin B12 Deficiency in the South of Jordan: A Possible Geographical Correlation

Janti Qar[1], Mazhar Salim Al Zoubi[2], Laila Alazzam[1], Bahaa Al-Trad[1], Ayman AlZu'bi[2], Manal Issam AbuAlarja[2], Khalid M. Al-Batayneh[1]

[1]Department of Biological Sciences, Faculty of Science, Yarmouk University, Irbid, Jordan; [2]Department of Basic Medical Sciences, Faculty of Medicine, Yarmouk University, Irbid, Jordan

Abstract. Vitamin B12 deficiency has been associated with certain serious disorders such as megaloblastic anemia as well as cardiovascular, neurological, and psychological disorders. Vitamin B12 deficiency is a worldwide health concern and a severe health problem in certain developing countries. In the current study, we aimed to evaluate the situation of vitamin B12 and estimate the prevalence of vitamin B12 deficiency in southern Jordan. The study population included 281 participants from Aqaba city: 71.2% females and 28.8% males with a mean age of 30.01±13.5. The results showed that the average vitamin B12 level was 277.34±103.70 pg/mL. The study population showed that 69 (24.6%) participants had a vitamin B12 deficiency. The current findings showed a lower frequency of vitamin B12 deficiency in the studied population compared to the rest of Jordan, especially in the north. The females reported a significantly higher frequency of vitamin B12 deficiency (29.0%) compared to the male group (13.6%). In addition, the CBC parameters showed a significant association between the level of vitamin B12 and Hb, RBC, PCV, and platelet count with a *p*-value = 0.044, <0.001, 0.021, 0.025, respectively. On the other hand, the results showed a lack of association between the vitamin B12 level and MCV and age group. In conclusion, the current study supports our previous findings regarding the correlation between geographical locations and the deficiency of vitamin B12. These findings can be attributed to the lifestyle and nutrient situation in Aqaba city which relies more on seafood. Moreover, other factors such as genetic predisposition can not be excluded as an association factor. Therefore, more studies are required to evaluate vitamin B12 levels in Jordan at a higher scale that will include genetic analysis as well as food habits.

Key words: Vitamin B12, Cobalamin, Vitamin Deficiency, Geographical location.

Introduction

Vitamin B12 (Cobalamin; Cbl) is a water-soluble molecule characterized by its chemical complexity, large size, and cobalt ion which is centrally located in the corrin ring (1). Vitamin B12 is essentially obtained from dietary sources such as red meat, dairy products, eggs, and fish (2-4). Absorption and transportation of vitamin B12 are processes that occur by hydrophobic binding protein carriers including haptocorrin (HC), intrinsic factor (IF), and transcobalamin (TCN) (5-7). Around 80% of cobalamin is carried by inactive haptocorrin (or transcobalamin I) and 20% is transported by active TCN II in the bloodstream (8-10). The deficiency of vitamin B12 is attributed to many factors including insufficient dietary supply, malabsorption, and deficient absorption cases (11-15). In addition, many risk factors are related to vitamin B12 deficiency including genetic predisposition, age, weight, gender, and ethnic variations (16-19).

Vitamin B12 deficiency is most prevalent in fatigue, memory impairment, skin pallor, skin hyperpigmentation, glossitis, and severe hematological, neurological, and psychiatric disorders (20-26). Vitamin B12 has a vital role in many processes including the synthesis of nucleic acids, regulating the metabolism of the macronutrients (proteins, fats, and carbohydrates), as well as some neurological functions (27-31). Moreover, vitamin B12 plays a crucial role in myelin biosynthesis, manufacturing, and development of erythrocytes (hematopoiesis) (11, 32, 33). Therefore, vitamin B12 deficiency is associated with serious neurological, hematological, and metabolic disorders (28, 34-37). Vitamin B12 deficiency is most prevalent in the elderly, children, pregnant women, nursing mothers, and vegetarians (38-41).

Vitamin B12 deficiency is a worldwide concern (16, 42, 43). Notably, vitamin B12 deficiency is not prevalent in wealthy countries except in the elderly, compared to developing countries where the prevalence of vitamin B12 deficiency can reach more than 50% of the population (16, 42, 44-49). For instance, the prevalence of vitamin B12 deficiency in the United States and the United Kingdom is approximately 6% in people under the age of 60 and about 20% of those over 60 years, whereas countries in Latin America have a deficiency incidence of approximately 40% (50-56). In Jordan, vitamin B12 deficiency is a considerable health problem (57), where the prevalence varies according to the study population in the last decades (57-59). In different studies in Jordan, vitamin B12 deficiency was estimated at different prevalence (16–50%) with the highest prevalence in the elderly above 55 years old (16, 17, 59-61).

Based on our previous findings, we proposed geographical location as an important factor in the estimation of vitamin B12 which may be related to socioeconomic status, lifestyle, and cultural background. We reported significant differences in the frequencies of vitamin B12 deficiency in different locations in Jordan. In particular, the results showed a lower frequency of vitamin B12 deficiency in the southern parts of Jordan compared to the northern cities (17). Consequently, the current study aimed to evaluate vitamin B12 levels among the Jordanian population in Aqaba city (the most southern city) and to assess the prevalence of vitamin B12 deficiency in Aqaba's Jordanian population. This study will complete the portrait of the national situation of vitamin B12 levels.

Materials and Methods

Study Design

This retrospective study was performed in the most southern part of Jordan (Aqaba province). Venous blood samples were collected from different hospitals in Aqaba, with a total number of 281 samples. Written informed consent was obtained from all participants, and the data collected were related to location, gender, age, and general health of the participants who were living in Aqaba. Exclusion criteria included subjects who showed diseases that lead to cobalamin deficiency such as peptic ulcer, gastrointestinal problems, pregnancy, diabetes, and individuals under vitamin B12 supplementation.

Blood Sampling

Blood samples were collected from the participants at the hospitals in the Aqaba Governorate, then serum was isolated and preserved at -70°C until use to measure the level of vitamin B12 and further study according to standard procedures using commercially available kits. Laboratory analyses were performed at hospital laboratories in Aqaba, Jordan. The level of vitamin B12 was assessed based on sandwich enzyme-linked immunosorbent assay technology, according to the manufacturer's protocol (Cyanocobalamin (Vitamin B12) ELISA Kit, Abbexa, UK).

Statistical Analysis

All the statistical analysis used to evaluate the prevalence of vitamin B12 deficiency in Aqaba was done using SPSS software version 25. Categorical data are expressed in frequency and percentages, scale data are expressed in mean ±SD, chi-square test of independence was used to examine the association between categorical data. Pearson's correlation was used to analyze the correlation between scale data, an

independent t-test was used for vitamin B12 mean differences between both genders, *P*-values < 0.05 were considered statistically significant.

Results

Demographic characteristics of the study population

The study recruited 281 participants from Aqaba city; including 200 females (71.2 %) and 81 males (28.8 %). The ages ranged between 0 and 80 years with a mean age of 30.01±13.5 (31.9±16.49 for males and 29.3±12.05 for females), where the age group between 0-39 years was more prominent; 217(77.2%), as compared to the age group ≥40 years; 64(22.8%). The mean of vitamin B12 for the study population was 277.34±103.70 pg/mL.

The cutoff value of vitamin B12 level was set at 200 pg/mL, where the individuals with vitamin B12 levels less than 200 pg/mL were categorized as deficient. Based on this categorization the results showed that 212 (75.4%) of participants had a normal vitamin B12 level while 69 (24.6%) of them had a vitamin B12 deficiency. According to the gender groups, the females reported a significantly higher frequency of vitamin B12 deficiency (29.0%) compared to the male group (13.6%). In addition, the mean hemoglobin (Hb) level for participants was 12.66±1.78, whereas the mean of RBC for participants was 4.54±0.50 as shown in table 1.

Mean differences in vitamin B12 levels between males and females

The mean differences of serum vitamin B12 levels between males and females were determined by an independent sample t-test and the results are shown in table 2. Our study showed a statistically significant difference in the mean of serum vitamin B12 between genders (*P*-value = 0.002), wherein females, it was 265.09 ±106.35 pg/mL, and 307.59 ±90.75 pg/mL in males.

In addition, a Chi-Square independence test was used to examine the association between vitamin B12 deficiency and gender. The findings showed that there was a statistically significant association between gender and vitamin B12 deficiency (X^2 = 7.399, (*P*-value = 0.007)), which indicates that the females had a higher proportion of vitamin B12 deficiency compared to the males as shown in table 3 and figure 1.

Table 1. Demographic and clinical characteristics of the study population.

Variable	Frequency (%)
Male Female	81 (28.8) 200 (71.2)
< 40 years > 40 years	217 (77.2) 64 (22.8)
B12-serum (Mean ±SD*)	277.34±103.70
HB (Mean ±SD)	12.49±1.88
RBC(Mean ±SD)	4.51 ±0.50
MCV(Mean ±SD)	83.26±6.45
PCV(Mean ±SD)	37.72±4.82
B12 category Deficiency < 200 pg/mL Normal ≥ 200 pg/mL	 69 (24.6) 212 (75.4)

* SD=Standard deviation

Table 2. Independent t-test results of serum B12 between males and females.

Variable	N	Serum B12 (Mean±SD)	T-value	df	P-value
Female	200	265.09 ±106.35	3.161	279	**0.002**
Male	81	307.59 ±90.75			

Table 3. Prevalence of vitamin B12 deficiency in both genders (females and males).

Variable	Vitamin B12 level		Total	*P*-value
	< 200 pg/mL	≥ 200 pg/mL		
Females	58 (29.0)	142 (71.0)	200	**0.007**
Males	11 (13.6)	70 (86.4)	81	
Total	69	212	281	

Association between age group and vitamin B12 level

The current study reported a lack of association between vitamin B12 deficiency and age groups (< 40, ≥ 40 years) according to the chi-square test (X^2 = 2.428, (*P*-value = 0.119)) (Table 4). An independent sample t-test was also used to test the mean

differences of vitamin B12 concerning age groups, and the results showed no statistical differences between them (*P*=0.661) (Table 5).

Association between complete blood count (CBC) parameters and serum vitamin B12

The association between vitamin B12 and complete blood count (CBC) parameters was analyzed using Pearson's correlation, and the results revealed that there was a statistically significant correlation between Hb level and vitamin B12 level as shown in table 6. In addition, a statistically significant correlation was observed between red blood cells (RBC) and vitamin B12 levels (r= 0.311, *P*<0.001). Pearson product-moment showed there was a statistically significant positive correlation between serum vitamin B12 and packed cell volume (PCV) (R=0.192, *P*=0.021), vitamin B12 serum and platelets (PLT) (R=0.193, *P*=0.025), while no statistically significant correlation was found with mean cell volume (MCV) (R= - 0.080, *P*=0.354) as illustrated in table 6.

The relationship between geographical location and vitamin B12 levels.

The results of this study support the previous findings for the four regions in Jordan; Irbid, Karak, Tafila, and Ma'an, where the percentages of vitamin B12 deficiency in these regions were 41%, 35%, 27% and 26% respectively (57). The current study evaluated vitamin B12 deficiency in Aqaba, which was 24.6%. Figures 2 and 3 illustrate a comparison between the frequencies of vitamin B12 deficiencies in five regions that cover Jordan from north to south.

Discussion

In the Middle East, different countries have reported a high prevalence of vitamin B12 deficiency that resulted in pernicious anemia [94]. Similarly, the

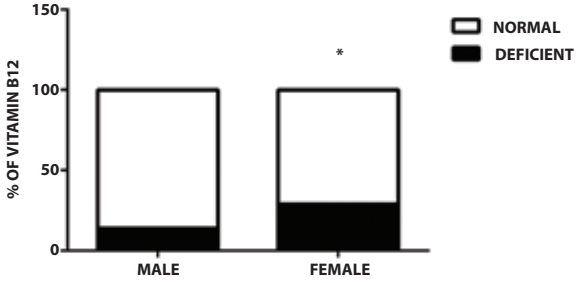

Figure 1. Frequency of vitamin B12 deficiency in females and males in Aqaba. Vitamin B12 deficiency in females is significantly more prevalent in females compared to males.

Table 6. Pearson correlation between CBC parameters and serum B12.

Variable		R	*P*-value
Serum B12 (pg/mL)	**HB** (g/dL)	0.167	**0.044**
	RBCs (uL)	0.311	**< 0.001**
	MCV (fl)	-0.080	0.354
	PCV (%)	0.192	**0.021**
	PLT (uL)	0.193	**0.025**

Table 4. Association between B12 deficiency and age groups.

Age group	Vitamin B12 category		Df	Chi-square	P-value
	Deficiency	Normal			
< 40 years	58 (26.7%)	159 (73.3%)	1	2.428	0.119
≥ 40 years	11 (17.2%)	53 (82.8%)			

Table 5. Mean differences in vitamin B12 levels in the age group.

Age groups		N	Mean ±SD	T-value	*P*-value
Serum B12	**< 40 years**	217	276.11 (±110.24)	0.439	0.661
	≥ 40 years	64	281.51 (±78.13)		

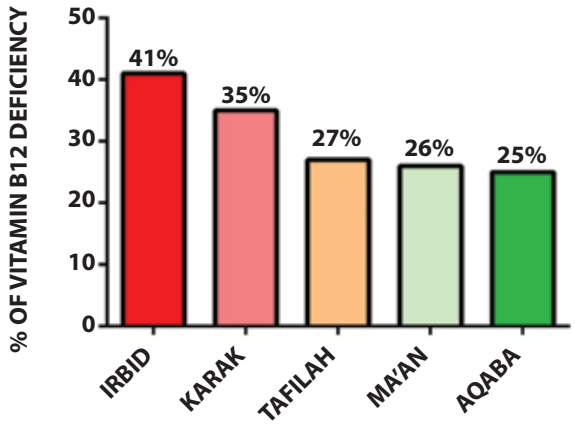

Figure 2. The frequency of vitamin B12 deficiency in different regions in Jordan (Irbid is representing the most northern part of Jordan while Aqaba is the most southern part of Jordan).

Figure 3. Heat map of Jordan according to the frequency of vitamin B12 deficiency. (Irbid, Karak, Tafilah, Ma'an, and Aqaba). Red = 41 %, Pink = up to 48 %, Orange = 35 %, Light green = 27 %, Dark green = 26 % and light blue = 25 % of vitamin B12 deficiency. The Dark blue area is not investigated.

deficiency of vitamin B12 in Jordan is a health concern that needs to be considered seriously. Previous studies showed differences in the prevalence of vitamin B12 deficiency in Jordan. For instance, in a study that included two different populations, the researchers reported 50.8% of vitamin B12 deficiency among the Arab population and 46.9% among Circassians (62). Other studies reported 10% (58), 24% (57), 27% (63), 30% (16) and 44.6% (64) of vitamin B12 deficiency. The variation in the prevalence can be attributed to the differences between the study populations and age groups (16, 57-61).

In a recent study, it was shown that vitamin B12 deficiency varies in different provinces of Jordan; Irbid, Karak, Tafilah, and Ma'an (57). In this retrospective study, we evaluated vitamin B12 levels in a Jordanian population in Aqaba city and correlated them with gender, age, and CBC parameters. The current study reported a 24.6 % vitamin B12 deficiency in the Aqaba province population. These findings support our previous report detailing the trend of vitamin B12 deficiency according to the geographical location in Jordan. Additionally, the results reported a significant difference between the means of vitamin B12 levels in males and females, 307.59 ±90.75, 265.09 ±106.35, respectively (p = 0.002). In particular, females showed a higher frequency of vitamin B12 deficiency (29%) compared to the male group (14%). Inconsistently with a previous study, the researchers showed that males had a higher frequency of vitamin B12 deficiency than females in Arab and Caucasian populations (62). However, there is some evidence that females have a higher frequency of vitamin B12 deficiency than males (65). Contrary, in another study, a higher percentage of vitamin B12 deficiency in males was found (15.2%) compared to females (11.5%) (66). Nevertheless, different findings have reported a lack of relationship between gender and vitamin B12 level (60). Therefore, other risk factors have to be considered in addition to gender, such as genetic predisposition, and physiological differences, and pregnancy frequency.

The current findings did not report a difference in the vitamin B12 deficiency between age groups; < 40 years and > 40 years (P=0.119), which is consistent with the results of a previous study (62). On the other hand, most studies showed a significant association between vitamin B12 deficiency and age (54, 63, 67, 68). In another previous survey that evaluated vitamin B12 deficiency in Jordan for participants whose ages ranged between 19 and 90, the study concluded that mean serum vitamin B12 levels rise with age, however,

individuals aged < 40 years had the lowest vitamin B12 levels among all ages, which is consistent with our findings (16). Nevertheless, the deficiency of vitamin B12 may commonly affect the elderly. Therefore, the lack of association between the age group and vitamin B12 deficiency can be attributed to the size of the study population from each group.

Herein, the level of vitamin B12 showed a significant association with the level of Hb (P = 0.044). Contrary, Qutob et al have shown that there was no correlation between vitamin B12 status and Hb (p=0.545) (63). Additionally, we observed a statistically significant positive correlation between RBCs count and vitamin B12 level (r= 0.354, p<0.001). Metabolically, vitamin B12 is an essential cofactor that results in normal blood cell maturation. Macrocytosis is a cause of ineffective erythropoiesis due to vitamin B12 or folate deficiency (69-72). Therefore, our results support the role of vitamin B12 in the erythropoietic process. Moreover, PCV and platelet count showed a significant association with the vitamin B12 level in the studied population. On the other hand, our findings did not show any significant association between the level of vitamin B12 and the MCV. On the contrary, many studies confirmed the inverse relationship between vitamin B12 level and MCV (63, 73, 74). An elevated MCV refers to the average red blood cell volume that may arise with or without reduced hemoglobin levels (65). Overall, pernicious anemia leads to vitamin B12 deficiency and results in producing abnormal and unhealthy RBCs, which means fewer functional erythrocytes (75-77).

The current study also aimed to complete the portrait of the prevalence of vitamin B12 deficiency in Jordan. In a previous study, we evaluated the prevalence of vitamin B12 deficiency in several regions, starting from the northern part of Jordan, Irbid (41%), Karak (35%), Tafilah (27%) and Ma'an (26%) where we moved in a southerly direction (57). Here, we reached the most southern part of Jordan, Aqaba, which was reported to have a 24.6% vitamin B12 deficiency. This study confirms the association between the geographical location and the frequency of vitamin B12 deficiency in Jordan (57). Fora and Mohammed reported similar observations in the north of Jordan (48%), where their study emphasized high vitamin B12 deficiency in northern regions (61). Moreover, southern regions had reported lower vitamin B12 deficiency than central regions (Amman), and Hakooz et al (2006) reported lower rates of vitamin B12 in Amman (48.5%) (62). However, the current results showed a similar mean level of vitamin B12 in Aqaba's population compared to Irbid's population and a lower level of vitamin B12 compared to the other parts of Jordan (Middle-South). Overall, the current study showed that the deficiency of vitamin B12 in Aqaba is lower than in other parts of Jordan.

Conclusions

To our knowledge, this is the first study to determine the prevalence of vitamin B12 deficiency in Aqaba province. Vitamin B12 levels were strongly and significantly correlated with gender and Hb, RBCs, PCV and platelet count with non-significant relation to age and MCV. Additionally, the study confirmed that Aqaba, like other southern regions in Jordan, is characterized by a lower frequency of vitamin B12 deficiency compared to the other regions in Jordan. Further studies are needed in other regions of Jordan, such as the eastern regions (Mafraq and Azraq) to complete the national portrait of the vitamin B12 level in Jordan. In addition, governmental measures are highly advised to reevaluate vitamin B12 levels in a cross-sectional study and establishment an appropriate fortification program in the northern part of Jordan, especially in females.

Acknowledgments: We are very thankful to the Deanship of Scientific Research and Graduate Studies at Yarmouk University for their financial support to complete this work. We thank Mr. Adel Rababah for his help in the fine art production.

Conflict of Interest: All authors declare the lack of conflict of interest in this work.

References

1. Fedosov SN. Physiological and molecular aspects of cobalamin transport. Water Soluble Vitamins: Springer; 2012. p. 347-67.

2. Gille D, Schmid A. Vitamin B12 in meat and dairy products. Nutrition reviews. 2015;73(2):106-15.
3. Herbert V. Vitamin B-12: plant sources, requirements, and assay. The American journal of clinical nutrition. 1988;48(3):852-8.
4. Watanabe F. Vitamin B12 sources and bioavailability. Experimental biology and medicine. 2007;232(10):1266-74.
5. Kalra S, Li N, Yammani RR, Seetharam S, Seetharam B. Cobalamin (vitamin B12) binding, phylogeny, and synteny of human transcobalamin. Archives of biochemistry and biophysics. 2004;431(2):189-96.
6. Wuerges J, Garau G, Geremia S, Fedosov SN, Petersen TE, Randaccio L. Structural basis for mammalian vitamin B12 transport by transcobalamin. Proceedings of the National Academy of Sciences. 2006;103(12):4386-91.
7. Moll R, Davis B. Iron, vitamin B12 and folate. Medicine. 2017;45(4):198-203.
8. O'Leary F, Samman SJN. Vitamin B12 in health and disease 2010;2(3):299-316.
9. van der Linden IJ, Afman LA, Heil SG, Blom HJ. Genetic variation in genes of folate metabolism and neural-tube defect risk. Proceedings of the Nutrition Society. 2006;65(2):204-15.
10. Pitkin RM, Allen L, Bailey L, Bernfield M. Dietary Reference Intakes for Thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, Pantothenic acid, biotin and choline. Washington, DC. 2000.
11. Gröber U, Kisters K, Schmidt JJN. Neuroenhancement with vitamin B12—underestimated neurological significance 2013;5(12):5031-45.
12. Brito A, Habeych E, Silva-Zolezzi I, Galaffu N, Allen LHJNr. Methods to assess vitamin B12 bioavailability and technologies to enhance its absorption 2018;76(10):778-92.
13. Antony AC. Vitamin B 12 (Cobalamin) and Folate Deficiency. Concise Guide to Hematology: Springer; 2019. p. 37-48.
14. Ankar A, Kumar A. Vitamin B12 Deficiency (Cobalamin). StatPearls [Internet]: StatPearls Publishing; 2019.
15. Sukumar N, Rafnsson SB, Kandala N-B, Bhopal R, Yajnik CS, Saravanan PJTAjocn. Prevalence of vitamin B-12 insufficiency during pregnancy and its effect on offspring birth weight: a systematic review and meta-analysis 2016;103(5):1232-51.
16. El-Khateeb M, Khader Y, Batieha A, Jaddou H, Hyassat D, Belbisi A, Ajlouni KJAoN, Metabolism. Vitamin B12 deficiency in Jordan: a population-based study 2014;64(2):101-5.
17. Al Zoubi MS, Al-Batayneh KM, Al-Trad B, Haddad Y, Hussein E, Al Khateeb W, Aljabali AA, Bodoor K, Shehab M, Al Hamad M. Evaluation of Vitamin B12, Folate and Ferritin Serum Levels in Jordanian Population. Journal of nutritional science and vitaminology. 2019;65(4):309-17.
18. Al-Batayneh KM, Al Zoubi MS, Al-Trad B, Hussein E, Al Khateeb W, Aljabali AA, Bodoor K, Shehab M, Al Hamad MA, Eaton GJ. Homologous G776G Variant of Transcobalamin-II Gene is Linked to Vitamin B12 Deficiency. International Journal for Vitamin and Nutrition Research. 2019.
19. Al-Batayneh KM, Al Zoubi MS, Shehab M, Al-Trad B, Bodoor K, Al Khateeb W, Aljabali AA, Al Hamad M, Eaton G. Association between MTHFR 677C> T polymorphism and vitamin B12 deficiency: a case-control study. Journal of Medical Biochemistry. 2018;37(2):141-7.
20. Clarke R, Refsum H, Birks J, Evans JG, Johnston C, Sherliker P, Ueland PM, Schneede J, McPartlin J, Nexo EJTAjocn. Screening for vitamin B-12 and folate deficiency in older persons 2003;77(5):1241-7.
21. Lee S, O'Dell D, Hohenstein J, Colt S, Mehta S, Erickson DJSr. NutriPhone: a mobile platform for low-cost point-of-care quantification of vitamin B 12 concentrations 2016;6:28237.
22. Moll R, Davis BJM. Iron, vitamin B12 and folate 2017; 45(4):198-203.
23. Stabler SPJNEJoM. Vitamin B12 deficiency 2013;368(2): 149-60.
24. Hunt A, Harrington D, Robinson SJB. Vitamin B12 deficiency 2014;349.
25. Bousselamti A, El Hasbaoui B, Echahdi H, Krouile YJTPAMJ. Psychomotor regression due to vitamin B12 deficiency 2018;30.
26. Dror DK, Allen LHJNr. Effect of vitamin B12 deficiency on neurodevelopment in infants: current knowledge and possible mechanisms 2008;66(5):250-5.
27. Pupić-Bakrač A, Pervan A, Pupić-Bakrač J, Končurat JJMFMF. Extremely severe vitamin B12 deficiency–case presentation and review of literature 2019;55(3):301-10.
28. Dror DK, Allen LH. Effect of vitamin B12 deficiency on neurodevelopment in infants: current knowledge and possible mechanisms. Nutrition reviews. 2008;66(5):250-5.
29. Hunt A, Harrington D, Robinson S. Vitamin B12 deficiency. Bmj. 2014;349.
30. Quinlivan E, McPartlin J, McNulty H, Ward M, Strain J, Weir D, Scott J. Importance of both folic acid and vitamin B12 in reduction of risk of vascular disease. The Lancet. 2002;359(9302):227-8.
31. Gröber U, Kisters K, Schmidt J. Neuroenhancement with vitamin B12—underestimated neurological significance. Nutrients. 2013;5(12):5031-45.
32. Gröber UJDM, Interactions D. Micronutrients: Metabolic tuning-prevention-therapy 2009;24(2-4):331-.
33. Koury MJ, Ponka P. New insights into erythropoiesis: the roles of folate, vitamin B12, and iron. Annu Rev Nutr. 2004;24:105-31.
34. Bernard MA, Nakonezny PA, Kashner TM. The effect of vitamin B12 deficiency on older veterans and its relationship to health. Journal of the American Geriatrics Society. 1998;46(10):1199-206.
35. Eastley R, Wilcock GK, Bucks RS. Vitamin B12 deficiency in dementia and cognitive impairment: the effects of treatment on neuropsychological function. International Journal of Geriatric Psychiatry. 2000;15(3):226-33.
36. Obeid R, Geisel J, Schorr H, Hübner U, Herrmann W. The impact of vegetarianism on some haematological parameters. European Journal of Haematology. 2002;69(5-6):275-9.

37. Miller JW, Garrod MG, Allen LH, Haan MN, Green R. Metabolic evidence of vitamin B-12 deficiency, including high homocysteine and methylmalonic acid and low holotranscobalamin, is more pronounced in older adults with elevated plasma folate. The American journal of clinical nutrition. 2009;90(6):1586-92.
38. Andrès E, Loukili NH, Noel E, Kaltenbach G, Abdelgheni MB, Perrin AE, Noblet-Dick M, Maloisel F, Schlienger J-L, Blicklé J-FJC. Vitamin B12 (cobalamin) deficiency in elderly patients 2004;171(3):251-9.
39. Green R, Allen LH, Bjørke-Monsen A-L, Brito A, Guéant J-L, Miller JW, Molloy AM, Nexo E, Stabler S, Toh B-HJNrDp. Vitamin B 12 deficiency 2017;3(1):1-20.
40. Pawlak R, Parrott SJ, Raj S, Cullum-Dugan D, Lucus D. How prevalent is vitamin B12 deficiency among vegetarians? Nutrition reviews. 2013;71(2):110-7.
41. Pawlak R, Lester S, Babatunde T. The prevalence of cobalamin deficiency among vegetarians assessed by serum vitamin B12: a review of literature. European journal of clinical nutrition. 2014;68(5):541-8.
42. Carmel RJArom. Current concepts in cobalamin deficiency 2000;51(1):357-75.
43. Stabler SP, Allen RH. Vitamin B12 deficiency as a worldwide problem. Annu Rev Nutr. 2004;24:299-326.
44. Hin H, Clarke R, Sherliker P, Atoyebi W, Emmens K, Birks J, Schneede J, Ueland PM, Nexo E, Scott JJA, ageing. Clinical relevance of low serum vitamin B12 concentrations in older people: the Banbury B12 study 2006;35(4):416-22.
45. Matthews JHJBsch. 12 Cobalamin and folate deficiency in the elderly 1995;8(3):679-97.
46. McLean ED, Allen LH, Neumann CG, Peerson JM, Siekmann JH, Murphy SP, Bwibo NO, Demment MW. Low plasma vitamin B-12 in Kenyan school children is highly prevalent and improved by supplemental animal source foods. The Journal of nutrition. 2007;137(3):676-82.
47. Siekmann JH, Allen LH, Bwibo NO, Demment MW, Murphy SP, Neumann CG. Kenyan school children have multiple micronutrient deficiencies, but increased plasma vitamin B-12 is the only detectable micronutrient response to meat or milk supplementation. The Journal of nutrition. 2003;133(11):3972S-80S.
48. Taneja S, Bhandari N, Strand TA, Sommerfelt H, Refsum H, Ueland PM, Schneede J, Bahl R, Bhan MK. Cobalamin and folate status in infants and young children in a low-to-middle income community in India. The American journal of clinical nutrition. 2007;86(5):1302-9.
49. Refsum H, Yajnik CS, Gadkari M, Schneede J, Vollset SE, Örning L, Guttormsen AB, Joglekar A, Sayyad MG, Ulvik A. Hyperhomocysteinemia and elevated methylmalonic acid indicate a high prevalence of cobalamin deficiency in Asian Indians. The American journal of clinical nutrition. 2001;74(2):233-41.
50. Langan RC, Goodbred AJJAfp. Vitamin B12 deficiency: recognition and management 2017;96(6):384-9.
51. Pfeiffer CM, Caudill SP, Gunter EW, Osterloh J, Sampson EJ. Biochemical indicators of B vitamin status in the US population after folic acid fortification: results from the National Health and Nutrition Examination Survey 1999–2000–. The American journal of clinical nutrition. 2005;82(2):442-50.
52. Pfeiffer CM, Johnson CL, Jain RB, Yetley EA, Picciano MF, Rader JI, Fisher KD, Mulinare J, Osterloh JD. Trends in blood folate and vitamin B-12 concentrations in the United States, 1988–2004. The American journal of clinical nutrition. 2007;86(3):718-27.
53. Campbell AK, Miller JW, Green R, Haan MN, Allen LH. Plasma vitamin B-12 concentrations in an elderly Latino population are predicted by serum gastrin concentrations and crystalline vitamin B-12 intake. The Journal of nutrition. 2003;133(9):2770-6.
54. Clarke R, Grimley Evans J, Schneede J, Nexo E, Bates C, Fletcher A, Prentice A, Johnston C, Ueland P, Refsum H. Vitamin B12 and folate deficiency in later life. Age and ageing. 2004;33(1):34-41.
55. Clarke R, Sherliker P, Hin H, Nexo E, Hvas AM, Schneede J, Birks J, Ueland PM, Emmens K, Scott JM. Detection of vitamin B12 deficiency in older people by measuring vitamin B12 or the active fraction of vitamin B12, holotranscobalamin. Clinical chemistry. 2007;53(5):963-70.
56. Allen LH. Folate and vitamin B 12 status in the Americas. Nutrition reviews. 2004;62(suppl_1):S29-S33.
57. Al Zoubi MS, Al-Batayneh KM, Al-Trad B, Haddad Y, Hussein E, Al Khateeb W, Aljabali AA, Bodoor K, Shehab M, Al Hamad MJJons, vitaminology. Evaluation of Vitamin B12, Folate and Ferritin Serum Levels in Jordanian Population 2019;65(4):309-17.
58. Abu-Samak M, Khuzaie R, Abu-Hasheesh M, Jaradeh M, Fawzi MJJoAS. Relationship of vitamin B12 deficiency with overweight in male Jordanian youth 2008;8(17):3060-3.
59. Barghouti F, Younes N, Halaseh L, Said T, Ghraiz SJE-EMHJ, 15, 853-860,. High frequency of low serum levels of vitamin B [12] among patients attending Jordan University Hospital 2009.
60. Alomari MA, Khabour OF, Gharaibeh MY, Qhatan RAJTP, sportsmedicine. Effect of physical activity on levels of homocysteine, folate, and vitamin B12 in the elderly 2016;44(1):68-73.
61. Fora MA, Mohammad MAJSmj. High frequency of suboptimal serum vitamin B12 level in adults in Jordan 2005;26(10):1591-5.
62. Hakooz N, Abu-Dahab R, Arafat T, Hamad M. A trend of low serum vitamin B12 in Jordanian adults from two ethnic groups in Amman. Jordanian Med J. 2006;40:80-7.
63. Qutob MS, Takruri HR, Barghouti FF. Evaluation of true vitamin B12 deficiency in a group of Jordanians aged 20-40 years visiting the Jordan University Hospital. Pakistan Journal of Nutrition. 2011.
64. Barghouti F, Younes N, Halaseh L, Said T, Ghraiz S. High frequency of low serum levels of vitamin B [12] among patients attending Jordan University Hospital. EMHJ-Eastern Mediterranean Health Journal, 15 (4), 853-860, 2009. 2009.

65. Perry M. Understanding vitamin B12 and folate deficiency. Practice Nursing. 2020;31(2):70-4.
66. Figlin E, Chetrit A, Shahar A, Shpilberg O, Zivelin A, Rosenberg N, Brok-Simoni F, Gadoth N, Sela BA, Seligsohn U. High prevalences of vitamin B12 and folic acid deficiency in elderly subjects in Israel. British journal of haematology. 2003;123(4):696-701.
67. Loikas S, Koskinen P, Irjala K, Löppönen M, Isoaho R, Kivelä S-L, Pelliniemi T-T. Vitamin B12 deficiency in the aged: a population-based study. Age and ageing. 2007;36(2):177-83.
68. Allen LH. How common is vitamin B-12 deficiency? The American journal of clinical nutrition. 2009;89(2):693S-6S.
69. Aslinia F, Mazza JJ, Yale SH. Megaloblastic anemia and other causes of macrocytosis. Clinical medicine & research. 2006;4(3):236-41.
70. Veit K, editor. Pseudothrombotic microangiopathy and vitamin B12 deficiency in pernicious anemia. Baylor University Medical Center Proceedings; 2017: Taylor & Francis.
71. Prueksaritanond S, Barbaryan A, Mirrakhimov AE, Liana P, Ali AM, Gilman AD. A puzzle of hemolytic anemia, iron and vitamin B12 deficiencies in a 52-year-old male. Case reports in hematology. 2013;2013.
72. Sahin K, Elevli M, Coskun C. Frequency of Anemia and Correlation with Mean Erythrocyte Volume in Children with B12 and Folate Deficiency 2019.
73. Oosterhuis W, Niessen R, Bossuyt P, Sanders G, Sturk A. Diagnostic value of the mean corpuscular volume in the detection of vitamin B12 deficiency. Scandinavian journal of clinical and laboratory investigation. 2000;60(1):9-18.
74. Vant Sant P, Küsters P, HARTHOORN-LASTHUIZEN E. Dependency of MCV and haemoglobin concentration on plasma vitamin B12 levels in relation to sex and age. Clinical & Laboratory Haematology. 1997;19(1):23-5.
75. Sun A, Chang JYF, Wang YP, Cheng SJ, Chen HM, Chiang CP. Do all the patients with vitamin B12 deficiency have pernicious anemia? Journal of Oral Pathology & Medicine. 2016;45(1):23-7.
76. Toh B-H. Pathophysiology and laboratory diagnosis of pernicious anemia. Immunologic research. 2017;65(1):326-30.
77. Abdulmanea A, Alsaeed A, Shaik A, AlGahtani F. Prevalence of pernicious anemia in patients with macrocytic anemia and low serum B12. Pakistan Journal of Medical Sciences. 2014;30(6):1218.

Correspondence
Mazhar Salim Al Zoubi, Ph.D
Department of Basic Medical Sciences,
Faculty of Medicine, Yarmouk University, Irbid, Jordan.
Email: mszoubi@yu.edu.jo.