High frequency of suboptimal serum vitamin B1 2 level in adults in Jordan

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ABSTRACT

Objective: To assess the prevalence of functional vitamin B12 deficiency status in an adult Jordanian seemingly healthy population.

Methods: We carried out this study in the Department of Physiology, Faculty of Medicine, Jordanian University of Science and Technology, Jordan from September 2004 to January 2005. We included a randomly selected sample of 216 healthy adult volunteers (male 124, female 92, aged 19-50 years) from north Jordan. We carried out the measurement of hematological variables by Micros 60-OT Analyzer. We measured the serum vitamin B12 by microparticle enzyme immunoassay and serum folate by ion-capture assay on an AxSYM Analyzer.

Results: We found suboptimal (<222 pg/ml) serum levels of vitamin B12 in 104 (48.1%) (male 64, female 40) subjects. The mean serum vitamin B12 level of male in all volunteers was 238.8 ± 97.3 pg/ml, not significantly different (p=0.24) from that of female (261.8 ± 101.6 pg/ml). The mean serum values of vitamin B12 of male in the suboptimal group (168.3 ± 36.4 pg/ml; range 90-221) were not significantly different from that of female (178.2 ± 27.5 pg/ml; range 113-217) (p=0.31). Of the suboptimal group, 30 subjects (28.8%) had hypersegmented neutrophils, 38 subjects (36.5%) had low mean corpuscular volume and 8 subjects (7.7%) had suboptimal serum folate levels.

Conclusion: This study revealed that there was a high frequency of suboptimal serum vitamin B12 level, which is an early sign of negative B12 balance.

Saudi Med J 2005; Vol. 26 (10): 1591-1595

Megaloblastic anemia is a disease caused by folic acid or vitamin B12 deficiency. The clinical manifestations are almost the same except neurological damage occurs in vitamin B12 deficiency. Vitamin B12 deficiency may take years to develop due to high storage in relation to the daily need of the body and efficient enterohepatic circulation of vitamin B12. Herbert[1] defined 4 stages in the development of vitamin B12 deficiency. Stages 1 and 2 comprise the state in which there are biochemical changes denoting functional deficiency of vitamin B12 before any obvious hematological and clinical manifestations appear. Stages 3 and 4 comprise the state of true vitamin B12 deficiency with obvious hematological, metabolic, clinical manifestations, or a combination of these conditions. Patients with vitamin B12 deficiency present with no symptoms or a variety of hematological and neuropsychiatric manifestations. Complaints vary from mild symptoms like lassitude or weakness to life-threatening situations like leucopenia or thrombocytopenia.[2] Vitamin B12 deficiency may be reflected on the digestive tract resulting in anorexia, glossitis, weight loss, and malabsorption. The classical hematological manifestations of vitamin B12 deficiency are anemia with macro ovalocytosis that occurs in the advanced stage of the disease. Other hematological manifestations may include hypersegmented neutrophils, anisopoikilocytosis, and elevated mean corpuscular volume (MCV).

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Received 16th March 2005. Accepted for publication in final form 26th June 2005.

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Neurological manifestations may vary depending which area of the nervous system is involved. Demyelination and axonal degeneration are the initial stages of neurological manifestation that will end with neuronal death.[3] Mental status may vary from mild excitability to dementia, depression, and psychosis.[4] Severe demyelination with paraparesis after nitrous oxide anesthesia is reported in patients with vitamin B12 deficiency.[5] Vitamin B12 deficiency has been connected also to Alzheimer's disease.[6] In addition, vitamin B12 deficiency plays an important role in developing serious diseases including cognitive dysfunction even without anemia.[1,7] It also induces hyperhomocystinemia, which is related to cerebral, coronary, and peripheral vascular disease.[8] The hematological, neurological and psychiatric disorders that arise from vitamin B12 deficiency are reversed by early diagnosis and adequate treatment.[9] Thus, early diagnosis of vitamin B12 deficiency is important to prevent further tissue damage and improve the condition.[3,4,6] Due to lack of information in Jordan on status of vitamin B12, it seemed desirable to see how serum vitamin B12 in a sample of Jordanian population is distributed. Accordingly, in this study, we chose a random sample of adult volunteers from north Jordan, measured serum vitamin B12, and serum folate levels. The aim was to assess the vitamin B12 status in an adult population of north Jordan.

Methods. Two hundred and sixteen participants (male 124, female 92; age 19-50 years) were recruited by simple random sampling. Recruitment of volunteers was from university students, employees, labors, and homemakers. A general questionnaire that includes dietary habit, socio-economic status, and medical history was used. Those who were strict vegetarians, smokers, taking food or multivitamin supplements, anticonvulsants, antimetabolites and antiviral drugs were excluded. Pregnant, lactating or on oral contraceptive women were also excluded. The Human Research Committee at Jordan University of Science and Technology approved the study. Informed written consent obtained from all participants before being admitted into the study. After fasting for 12 hours, 15 ml of blood was collected by venipuncture at midmorning from eligible subjects. Samples for vitamin B12 determination were placed immediately on ice and transferred in a cooled container to the laboratory within one hour. The EDTA was used as an anti-coagulant for hematological determinations. Erythrocyte counts, hemoglobin, packed cell volume (PCV), and mean corpuscular volume (MCV) were determined using Micros 60-OT Analyzer (ABX Diagnostix, France). The samples for vitamin B12 determination collected in tubes containing no anticoagulants were directly centrifuged at 3000 X g for 15 minutes at 20°C to separate serum, which was stored at -70°C, for further analysis. Serum vitamin B12 was measured by microparticle enzyme immunoassay and serum folate by ion-capture assay on an AxSYM Analyzer (Abbot Diagnostics, Germany). Serum iron levels were measured using Ferrimat kit (BioMerieux sa, France). The neutrophil lobe counts were performed on peripheral blood films stained with Wright stain. One hundred neutrophils were examined for each participant. Neutrophil hypersegmentation was defined as >5% of the neutrophils with 5 or the presence of at least one neutrophil with 6 or more lobes.[1] All the experimental data were subjected to Student's t-test for independent samples for vitamin B12 level. According to the reference ranges provided by the manufacturer, the data of serum vitamin B12 level were divided into 2 groups, suboptimal (<222 pg/ml) and optimal (≥222 pg/ml). Independent t-test statistics were applied to test the mean difference between males and females on the suboptimal group. Significance was considered at $p<0.05$.

Results. **Table 1** summarizes the description of the variables measured. The mean age of men was 31.3 years (range 19-48 years) and of women 29.7 years (range 19-50 years). The mean value of serum vitamin B12 of males was lower than females of all participants (p=0.24). Insignificant higher mean serum folate values were observed in men than in women (p=0.73). The mean MCV was within normal range for both males and females. A serum vitamin B12 value of less than 222 pg/ml was considered as a low value according to the manufacturer's recommendation. Many studies have put a lower limit of normal for vitamin B12 serum concentration approximately 200 pg/ml.[10-13] Based on the above recommendations, we divided the subjects into 2 groups: optimal group (subjects with serum vitamin B12 concentration ≥222 pg/ml and suboptimal group (subjects with serum vitamin B12 concentration <222 pg/ml). The hematological and biochemical values of optimal and suboptimal groups are shown in **Table 2**. The mean of serum vitamin B12 concentration in the entire suboptimal group (n=104) was 172 ± 1 pg/ml. There was no statically significant difference between male and female values in this group. Of 104 subjects in suboptimal group, 30 subjects (28.8%) had hypersegmented neutrophils, 38 subjects (36.5%) had low MCV, and 8 subjects (7.7%) had suboptimal serum folate concentration (**Table 3**).

Discussion. In this study, we aimed to assess the status of vitamin B12 in a sample of seemingly healthy individuals in north Jordan, and we found a high frequency of suboptimal serum vitamin B12 in that sample. One hundred and four participants had

Table 1 - Mean hematological parameters and serum biochemical values for vitamin B12, folate, and iron in total sample.

Parameters	Male (n=124)		Female (n=92)	
	Mean ± SD	Range	Mean ± SD	Range
Hb	14.5 ± 0.9	12.8 - 17.3	12.8 ± 0.6	12 - 15.2
RBC	5.3 ± 0.4	4 - 6.4	4.6 ± 0.5	3.8 - 6. 6
PCV	43 ± 3	37.2 - 52	37.1 ± 4.4	32.8 - 42.8
MCV	81.6 ± 5.9	71 - 95	80.8 ± 7.2	61 - 94
S. Fol	5.9 ± 2	2.2 - 10.2	5.8 ± 2.5	2.9 - 13.8
S. B12	238.8 ± 97.3	90 - 515	261.8 ± 101.6	113 - 500
SI	25.7 ± 3.2	20.7 - 28.1	20.2 ± 5.1	14.7 - 25.8

SD - standard deviation, Hb - hemoglobin (g%), RBC - red blood corpuscles (million/cmm), PCV - packed cell volume (%), MCV - mean corpuscular volume (fl), S. Fol - serum folate (ng/ml), S. B12 - serum vitamin B12 (pg/ml), SI - serum iron (mmol/l).

Table 2 - Mean hematological variables and serum biochemical values for optimal and suboptimal groups (mean ± SD).

Parameters	Optimal		Suboptimal	
	Male n=60	Female n=52	Male n=64	Female n=40
Hb	14.4 ± 0.9	12.7 ± 0.5	14.5 ± 0.9	12.7 ± 0.8
RBC	5.2 ± 0.5	4.6 ± 0.4	5.3 ± 0.4	4.6 ± 0.6
PCV	42.7 ± 2.9	37.3 ± 1.9	43.2 ± 3	36.9 ± 2.3
MCV	81.4 ± 6.6	80.4 ± 7.5	81.7 ± 5.1	81.3 ± 6.9
S. Fol	5.8 ± 2	6.1 ± 2.7	6 ± 2.1	5.5 ± 2.2
S. B12	314 ± 84.9	326.2 ± 90.2	168.3 ± 36.3	178.2 ± 27.4
SI	24.5 ± 3.5	19.6 ± 4	21.2 ± 3.7	17.4 ± 3.3

SD - standard deviation, Hb - hemoglobin (g%), RBC - red blood corpuscles (million/cmm), PCV - packed cell volume (%), MCV - mean corpuscular volume (fl), S. Fol - serum folate (ng/ml), S. B12 - serum vitamin B12 (pg/ml), SI - serum iron (mmol/l).

suboptimal serum vitamin B12 concentrations comprising 48.1% of all participants. Despite low levels of serum vitamin B12 found in almost half of our healthy population sample, we observed normal hematological values and absence of obvious clinical manifestation of vitamin B12 deficiency in this sample. This may indicate that the deficiency of vitamin B12 status in this population sample is of stage 1 and 2 according to Herbert's classification.[1] Similar to our finding, Refsum et al[14] observed a high incidence (47%) of low serum vitamin B12 in Asian-Indians. Gielchinksky et al[13] reported a high prevalence of low serum vitamin B12 level in 31% of adult healthy Ashkenazi Jews, and in 33% of healthy Syrian volunteers.[15] Reports of this high prevalence of low vitamin B12 status also came from Mexico, Venezuela and Guatemala.[16-18] On the contrary, other studies found low prevalence of vitamin B12 deficiency in healthy adults. A study carried out in France by de Carvalho et al[19] showed that less than 5% of subjects had values in the range of major vitamin B12 deficiency. Similarly, 2.4% of the adult healthy male constructor workers in Bangkok[20] showed vitamin B12 deficiency. Warta-nowicz et al[21] carried out a study in Poland on healthy women aged 19-30 years and revealed no risk of vitamin B12 deficiency. We can attribute the difference in the frequency of low serum vitamin B12 level reported by different authors to different cutoff values of serum vitamin B12 in definition of the deficiency, the method used to measure serum vitamin B12 level, geographical distribution, or ethnicity.[13,22] Gielchinsky et al[13] clearly demonstrated the effect of ethnicity on the prevalence of functional B12 deficiency.

The deficiency of vitamin B12 and folic acid impairs DNA synthesis by reducing the rate of cell division. Fewer cellular divisions cause red blood cells to become larger than normal.[23] Approximately 63% of the subjects in the suboptimal group had an MCV within normal range and none of the subjects

Table 3 - Hypersegmented neutrophils, mean corpuscular volume, and serum folate parameters related to gender in optimal and suboptimal groups.

Serum B12	Male						Female					
	Hypersegmented neutrophil		Mean corpuscular volume (fl)		Serum folate (ng/ml)		Hypersegmented neutrophil		Mean corpuscular volume (fl)		Serum folate (ng/ml)	
	Absent	Present	≥80	<80	<3.1	≥3.1	Absent	Present	≥80	<80	<3.1	≥3.1
Optimal ≥222 (319.7±87.2 pg/ml)	54	6	60	0	2	58	40	12	52	0	0	52
Suboptimal <222 (172.1±33.4 pg/ml)	50	14	40	24	6	58	24	16	26	14	2	38

in this group showed evidence of macrocytosis (MCV >100 fl). Other conditions that may cause small red blood cells like thalassemia may mask the macrocytosis.[24] Approximately 6% of people living in north Jordan have thalassemia.[25] Haltmayer et al[26]showed that there was no significant correlation of MCV with serum vitamin B12. Other researchers found that MCV in women increased only significantly at serum vitamin B12 level below 145 pg/ml.[10] It seems that vitamin B12 deficiency in our study was not severe enough to cause macrocytosis or other conditions that cause decrement in RBC size mask the macrocytosis. The serum folate level in the suboptimal group is largely normal except for 8 subjects whose serum folate level was not far from the normal value. The normal level of serum folate in the suboptimal group may mask the manifestations of the vitamin B12 deficiency.[27] The hematological variables are almost normal in the suboptimal group and not differ from that of optimal group (**Table 2**). We can attribute this either to normal serum folate and serum iron concentrations in the suboptimal group, or vitamin B12 deficiency is not severe enough to produce hematological changes. We noted hypersegmentation of neutrophils in 29% of the suboptimal group and in 16% of the optimal group. Previous studies reported neutrophils nuclear hypersegmentation in both iron deficiency anemia[28] and vitamin B12 deficiency.[29] The presence of neutrophils hypersegmentation in some subjects in the optimal group could be due to the coexistence of the early stage of iron deficiency in these subjects. Sipahi noted neutrophils hypersegmentation in 9% of children with normal level of serum vitamin B12.[28] The mean level of serum vitamin B12 level is almost close to those reported by Ardawi et al[30] in Saudis. Our volunteers reported an omnivorous diet, however, we could not exclude inadequate dietary intake, decreased vitamin B12 bioavailability, and other factors leading to low serum vitamin B12 levels in the suboptimal group. In addition, and due to low socioeconomic status, the major diet constituent among the Jordanian population is carbohydrates, and meat is of a minority group. Over the last few decades, the Jordanian population started to change their dietary habits from being dependent on homemade to manufactured food. The change in dietary habits might be one of the causes for low serum level of vitamin B12. It has been proposed that the increase incidence of certain diseases in the Mediterranean population such as diabetes, coronary artery diseases, and some types of cancer may be attributed to changes in dietary habits in these populations.[31] The significance of the high frequency of suboptimal serum levels of vitamin B12 in the participants remains unknown. Potential functional consequences of the low vitamin B12 status may occur, like poor growth, anemia, and neuropsychiatric manifestations including mood changes, and disturbances in sensory, motor, and cognitive functions.[3,32] Short-term memory and perception are substandard in the vitamin B12-deficient compared to the vitamin B12-adequate group in Guatemalan school children.[33] In addition, Wright[34] and Katka[35] reported the association of functional vitamin B12 deficiency with abnormality in humoral and cellular immunity. Consequently, the early diagnosis of functional vitamin B12 deficient subjects is important to prevent the possible occurrence of these complications. Depending on low hemoglobin, hematocrit, or high mean corpuscular volume in diagnosis of vitamin B12 deficiency will miss approximately 30% of adult cases.[36] Therefore, we must look for a functional B12 deficiency state.

In summary, we detected that almost half of the volunteers have suboptimal concentrations of serum vitamin B12 in the absence of clinical manifestations of megaloblastic anemia. This may have a health impact on these volunteers. We require further investigations for the definitive diagnoses of true B12 deficiency by measuring other variables that indicate tissue deficiency. This study focuses attention on low levels of vitamin B12 as a potential health problem in the community. To prevent vitamin B12 deficiency, we recommend annual screening of those who are at risk to develop vitamin B12 deficiency. This allows the tracking of minor declines in vitamin B12 status. We must consider the encouragement of people to eat fortified cereals, dairy products and fish to prevent vitamin B12 deficiency in the risky people. We also recommend following up the research to ascertain the extent of vitamin B12 deficiency in the Jordanian population.

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