High frequency of low serum levels of vitamin B_{12} among patients attending Jordan University Hospital

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ارتفاع معدَّل المستويات المنخفضة للفيتامين B_{12} بين المرضى في مستشفى الجامعة الأردنية

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الخلاصة: تهدف هذه الدراسة لتقدير وضع مستويات فيتامين B_{12} في المصل لدى المرضى في مستشفى الجامعة الأردنية في عمّان، مع دراسة العلاقة بينهم وبين المعطيات الديموغرافية والأمراض المزمنة والعادات الغذائية والمتثابتات الدموية والأعراض المتعلّقة بمستويات الفيتامين B_{12}. فمن بين 838 مريضاً استكملوا الاستمارات وأعطوا عينات من دمائهم كان لدى 44.6٪ منهم عوز في فيتامين B_{12} (أقل من 180 بيكوغرام/ ميلي لتـر)، ولدى 34.2٪ منهم نقص في الفيتامينات (180 – 300 بيكو غرام/ ميلي لتـر). وقد تـرافق عَوَز الفيتامين B_{12} مع اختلال الذاكرة، وانخفاض في تناول اللحم واتباع نظام غذائي نباتي صارم. وإن المعدَّل المرتفع لنقص مستوى فيتامين B_{12} يتطلَّب إعداد استـراتيجية لتصحيح هذه المشكلة في الأردن.

ABSTRACT This study aimed to estimate the status of serum vitamin B_{12} level in patients attending Jordan University Hospital in Amman, and to examine the relationship with demographic data, chronic illness, dietary habits, haematological parameters and symptoms related to vitamin B_{12} levels. A total of 838 patients completed a questionnaire and gave blood samples; 44.6% were vitamin B_{12} deficient (< 180 pg/mL) and 34.2% had hypovitaminosis (180–300 pg/mL). Vitamin B_{12} deficiency was associated with memory impairment, low meat intake and strict vegetarian (vegan) diets. The high frequency of low vitamin B_{12} warrants the development of a strategy to correct this problem in Jordan.

Fréquence élevée de faibles taux de vitamine B_{12} sériques chez les patients de l'hôpital universitaire de Jordanie

RÉSUMÉ Cette étude visait à estimer les taux de vitamine B12 sériques chez les patients de l'hôpital universitaire de Jordanie à Amman, et à examiner la relation avec les données démographiques, les maladies chroniques, les habitudes alimentaires, les paramètres hématologiques et les symptômes liés à ces taux. Au total, 838 patients ont rempli un questionnaire et ont été soumis à une prise de sang ; 44,6 % présentaient une déficience en vitamine B12 (< 180 pg/mL) et 34,2 % une hypovitaminose (180-300 pg/mL). La déficience en vitamine B12 était associée à des troubles de la mémoire, de faibles apports carnés et un régime végétarien strict (végétalien). La fréquence élevée de faibles taux de vitamine B12 justifie l'élaboration d'une stratégie visant à corriger ce problème en Jordanie.

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Introduction

Vitamin B_{12} is an essential vitamin derived from food of animal origin. The recommended average daily intake for adults is 2–4 μg [*1*]. The liver is the largest reservoir for B_{12} in the body; it stores up to 3 mg, which is sufficient for several years before the neurological and haematological sequelae of deficiency become evident [*2*]. B_{12} deficiency is a common but under-recognized disorder with a prevalence of 3%–40% in the adult population [*3–7*]. There is, however, considerable debate concerning the definition of the lower limits of vitamin B_{12} levels in healthy adults [*8,9*].

Prevention of micronutrient deficiency enhances human development and economic wellbeing. The rate of anaemia among women of childbearing age in Jordan is 32% and the rate among children is 20% according to the results of a joint study conducted by the Jordanian Ministry of Health, United Nations Children's Fund (UNICEF) and the World Health Organization (WHO).

The study highlights the importance of regular monitoring of micronutrient status in young children (< 5 years) and women of childbearing age (15–49 years) in Jordan. UNICEF and WHO embarked on a flour fortification campaign in 2002. As a result, bread has been fortified with iron and other micronutrients such as vitamin A.

We aimed to estimate serum vitamin B_{12} levels in patients attending clinics at Jordan University Hospital in Amman, and to examine any relationship between demographic data, chronic illness, dietary habits, haematological parameters and symptoms related to B_{12} levels.

Methods

All patients presenting to the family medicine clinic and the diabetic foot clinic at Jordan University Hospital from October 2005 to January 2006 were evaluated for vitamin B_{12} deficiency. Attendees at family medicine clinics are mostly healthy adults coming for minor ailments, acute problems or administrative reasons; a few have chronic illness. Patients attending the diabetic foot clinic are by definition suffering from a chronic illness. The 2 groups were chosen to ensure representation of both healthy and ill adults.

A total of 838 adults aged 18–78 years were included in the study (18 years was the cut-off). All persons attending the 2 clinics were offered the chance to be included in the study. Exclusion criteria were pregnant females, patients currently on vitamin B_{12} or any other multivitamin supplements and those on anticonvulsant drugs. Informed consent was obtained from each participant and the study was approved by the scientific research committee at the Faculty of Medicine, University of Jordan.

The data collected included age, sex, presence of any chronic illness and dietary habits. Chronic illness was defined as any disease lasting more than 3 months, such as diabetes mellitus, hypertension, coronary artery disease, hyperlipidaemia and peptic ulcer disease, confirmed by a review of medical records and medication intake.

Dietary habits were assessed by asking respondents to state quantity and frequency for consumption of: meat and chicken (1 serving = 70 g), eggs (1 serving = 1 egg) and dairy products (1 serving = 8 oz or 1 cup of milk or yogurt or 35 g white cheese), and whether they were strict vegetarians (i.e. vegan, eating a diet of exclusively vegetable origin).

Participants were also asked if they suffered from tiredness, fatigue, numbness or paraesthesias, impaired memory, irritability or depression to assess symptoms of possible B_{12} deficiency.

Fasting blood samples were collected from each patient for the measurement of haemoglobin level (normal: females 14 ± 2 g/dL, males 16 ± 2 g/dL; low: females < 12 g/dL, males < 14 g/dL), ferritin level (normal: females 14–150 µg/L, males 17–230 ug/L), folic acid level (normal: > 2.5 ng/mL), mean corpuscular volume (high: > 100 fL, normal: 80–100 fL, low: < 80 fL) and peripheral blood smear (normal; hypochromic; hypersegmented).

Serum vitamin B_{12} level was measured by chemiluminescent microparticle immunoassay (Abbot Laboratories, Illinois, United States of America). The range of the assay is 180–1162 pg/mL. Using the reference range provided by the manufacturer, participants were divided into 3 groups according to B_{12} level: normal (> 300 pg/mL), hypovitaminosis (180–300 pg/mL), vitamin B_{12} deficiency (< 180 pg/mL).

The data were analysed using *SPSS*, version 11.0 for Windows. Cross-tabulation and chi-squared tests were used to evaluate the variables that can influence vitamin B_{12} level or predict the presence of B_{12} deficiency. Statistical significance was set at $P < 0.05$.

Results

The results were based on analysis of 838 patients—280 (33.4%) males and 558 (66.6%) females—seen at the family medicine and diabetic foot clinics at Jordan University Hospital. The number of women was greater than men as they attend facilities more often than men as patients and as the companions of patients.

Table 1 shows the demographic features in relation to serum levels of vitamin B_{12}. Deficiency (< 180 pg/mL) was detected in 374 patients (44.6%). The frequency of B_{12} deficiency was 51.8% among the age group 18–24 years and was higher in men (52.1%) than in women (41.0%) ($P < 0.001$). B_{12} hypovitaminosis (180–300 pg/mL) was seen in 287 patients (34.2%), more in women (36.0%) than in men (30.4%).

There was no significant association between serum level of vitamin B_{12} and the clinical signs of B_{12} deficiency (fatigue, numbness), except for memory impairment, which was found in significantly more patients with B_{12} deficiency than other patients ($P < 0.05$) (Table 2). More patients with normal ferritin level were found to be B_{12}

Table 1 **Serum vitamin B_{12} levels according to age and sex**

Variable	Total	Normal B_{12}		B_{12} hypovitaminosis		B_{12} deficiency		*P*-value
	No.	No.	%	No.	%	No.	%	
Age (years)								
18–24	195	26	13.3	68	34.9	101	51.8	0.001
25–39	213	41	19.2	82	38.5	91	42.7	
40–54	250	57	22.8	75	30.0	119	47.6	
55–64	114	34	29.8	33	28.9	47	41.2	
> 64	54	17	31.5	24	44.4	13	24.1	
Missing	12	–	–	–	–	–	–	
Sex								
Female	558	128	22.9	201	36.0	229	41.0	0.008
Male	280	49	17.5	85	30.4	146	52.1	
Total	838	177	21.1	287	34.2	374	44.6	

deficient than those with low ferritin level (Table 3). There was no statistically significant relationship between serum levels of folic acid and B_{12} levels.

No significant association was found between vitamin B_{12} level and hypertension, coronary artery disease or peptic ulcer disease (Table 4). Patients with diabetes and hyperlipidaemia had significantly higher B_{12} levels than nondiabetic patients or patients with normal lipid profile ($P < 0.01$ and $P < 0.05$, respectively).

Low meat intake and strict vegetarian diets were associated with B_{12} deficiency ($P < 0.05$) (Table 5). Patients who ate meat less than twice per month were more likely to have vitamin B_{12} deficiency (56.4%) than those who ate meat daily (43.3%).

Discussion

Severe B_{12} deficiency (< 180 pg/mL) resulting in neurological and haematological manifestations is comparatively rare, especially in developed countries such as the United States of America and European countries [*10,11*]. However, suboptimal serum levels of vitamin B_{12} (hypovitaminosis) are common and the prevalence is rising among lactovegetarians and patients older than 60 years [*12*]. In North America the deficiency is particularly observed in elderly, institutionalized patients [*10,13*].

The frequency of vitamin B_{12} deficiency in this study was 44.6%. This is similar to a previous report from north Jordan on 216 healthy individuals by Fora and Mohammad where a suboptimal (< 222 pg/mL) serum level of B_{12} was observed in 48.1% of patients [*14*], and also similar to reports from Israel by Masalha et al. (49%) and Gielchinsky et al. (31%) [*15,16*]. Similar reports of high prevalence in this region came from Turkey (46.8%) [*17*] and the Islamic Republic of Iran (26.7%) [*18*]. These are higher than the reported global prevalence, which ranges from 3% to 40% of the adult population [*3–7*]. Some evidence suggests that this variation is mainly related to ethnic variations, dietary habits, geographical variation and intestinal malabsorption problems, or to different cut-off values for serum vitamin B_{12} level in the definition of deficiency [*17,18*].

Most reported studies have shown a gradual rise in the incidence of vitamin B_{12} deficiency with age. In our study, the highest frequency of deficiency was observed in

Table 2 **Symptoms related to vitamin B_{12} deficiency and serum vitamin B_{12} levels**

Variable	Total (*n* = 838)	Normal B_{12} (*n* = 177)		B_{12} hypovitaminosis (*n* = 287)		B_{12} deficiency (*n* = 374)		*P*-value
	No.	No.	%	No.	%	No.	%	
Fatigue								
Yes	306	76	24.8	108	35.3	122	39.9	0.05
No	532	101	19.0	179	33.6	252	47.4	
Numbness								
Yes	307	71	23.1	99	32.2	137	44.6	0.47
No	531	106	20.0	188	35.4	237	44.6	
Memory impairment								
Yes	235	61	26.0	66	28.1	108	46.0	0.02
No	603	116	19.2	221	36.7	266	44.1	

the age group 18–24 years (51.8%), while B_{12} hypovitaminosis was mostly observed in patients aged > 64 years (44.4%). These results were based only on B_{12} levels, without measuring methylmalonic acid and/or homocysteine, which was a limitation to this study.

We also noted that men had lower levels of vitamin B_{12} compared to women. The same result has been noted in other studies [*14,18*].

Our study demonstrated a negative correlation between diabetes and vitamin B_{12} deficiency ($P < 0.01$) and between hyperlipidaemia and B_{12} deficiency ($P < 0.04$). Diabetic patients and patients with hyperlipidaemia were less likely to have B_{12} deficiency.

Similar to the results reported by Fora and Mohammad from northern Jordan [*14*], the values of several haematological parameters were normal in most patients and there were no obvious clinical manifestations of vitamin B_{12} deficiency in this study. This could be explained by a mild and early form of B_{12} deficiency according to Herbert's classification [*19*].

Mild memory impairment was the only clinical manifestation correlated with vitamin B_{12} deficiency ($P < 0.02$). Reports of mental disturbances are frequent and are

Table 3 **Haematological parameters and serum vitamin B_{12} levels**

Variable	Total (*n* = 838)	Normal B_{12} (*n* = 177)		B_{12} hypovitaminosis (*n* = 287)		B_{12} deficiency (*n* = 374)		*P*-value
	No.	No.	%	No.	%	No.	%	
Haemoglobin level[a]								
Low	33	10	30.3	8	24.2	15	45.2	0.30
Normal	805	167	20.7	279	34.7	359	44.6	
Folic acid level[b]								
Low	10	3	30.0	4	40.0	3	30.0	0.62
Normal	827	174	21.0	283	34.2	371	44.8	
Ferritin level[c]								
Low	233	42	18.0	95	40.8	96	41.2	0.04
Normal	605	135	22.3	192	31.7	278	46.0	
Mean corpuscular volume[d]								
High	4	0	0.0	0	0.0	4	100.0	0.20
Normal	666	137	20.6	229	34.4	300	45.0	
Low	168	40	23.8	58	34.5	70	41.7	
Peripheral blood smear								
Normal	672	136	20.2	231	34.4	305	45.4	0.07
Hypochromic	94	30	31.9	29	30.9	35	37.2	
Hypersegmented	72	11	15.3	27	37.5	34	47.2	

[a]*Normal: females 14 ± 2 g/dL, males 16 ± 2 g/dL; low: females < 12 g/dL, males < 14 g/dL.*
[b]*Normal: > 2.5 ng/mL.*
[c]*Normal: females 14–150 µg/L, males 17–230 µg/L.*
[d]*High: > 100 fL; normal: 80–100 fL; low: < 80 fL.*

sometimes the only neurological manifestations of vitamin B_{12} deficiency [*1,2,20–22*].

We observed a 56.4% rate of vitamin B_{12} deficiency among patients getting less than 2 servings of meat per month compared with 43.3% in those who ate meat on a daily basis. This measure relied only on patients' estimates of the amount eaten, which is a limitation of the study, although we tried to minimize this by cross-referencing the servings they reported to culturally-determined servings in order to validate the amount of meat consumed. The validity and reliability of respondents' reporting has always been questioned. It is surprising that 43.3% of those who ate meat daily had vitamin B_{12} values < 180 pg/mL; this may be related to absorption problems, which needs further evaluation. In this study, vitamin B_{12} deficiency was also noted among 62.3% of strict vegetarians compared with 43.4% of nonvegetarians, suggesting that nutritional deficiency is an important cause of low serum levels of vitamin B_{12}.

The only current mass public health control and prevention of micronutrient deficiencies in Jordan depends on iron and vitamin A fortification of bread, in addition to dispensing multivitamin and mineral tablets weekly to students at public elementary schools.

This study looked at a sample drawn from attendees at 1 hospital in the capital city, so the results cannot be generalized to Jordan as a whole. However, the results may have important public health consequences and warrant further research in collaboration with the Jordanian Ministry of Health and WHO to evaluate B_{12} status in Jordan as a whole and to develop a strategy to correct this problem if similar results are found at a national level.

Table 4 **Presence of chronic diseases and serum vitamin B_{12} levels**

Variable	Total (*n* = 838)	Normal B_{12} (*n* = 177)		B_{12} hypovitaminosis (*n* = 287)		B_{12} deficiency (*n* = 374)		*P*-value
	No.	No.	%	No.	%	No.	%	
Diabetes mellitus								
Yes	95	31	32.6	30	31.6	34	35.8	0.01
No	743	146	19.7	257	34.6	340	45.8	
Hypertension								
Yes	157	43	27.4	51	32.5	63	40.1	0.10
No	681	134	19.7	236	34.7	311	45.7	
Coronary artery disease								
Yes	35	12	34.3	11	31.4	12	34.3	0.14
No	803	165	20.5	276	34.4	362	45.1	
Hyperlipidaemia								
Yes	93	28	30.1	33	35.5	32	34.4	0.04
No	745	149	20.0	254	34.1	342	45.9	
Peptic ulcer disease								
Yes	39	14	35.9	13	33.3	12	30.8	0.05
No	799	163	20.4	274	34.3	362	45.3	

Table 5 **Food consumption and serum vitamin B_{12} levels**

Variable	Total (*n* = 838)	Normal B_{12} (*n* = 177)		B_{12} hypovitaminosis (*n* = 287)		B_{12} deficiency (*n* = 374)		*P*-value
	No.	No.	%	No.	%	No.	%	
Meat[a]								
Daily	67	15	22.4	23	34.3	29	43.3	0.02
1–3 times/week	608	137	22.5	218	35.9	253	41.6	
< 2 times/month	163	25	15.3	46	28.2	92	56.4	
Chicken[a]								
Daily	79	19	24.1	25	31.6	35	44.3	0.44
1–3 times/week	681	143	21.0	241	35.4	297	43.6	
< 2 times/month	78	15	19.2	21	26.9	42	53.8	
Eggs[b]								
Daily	98	27	27.6	30	30.6	41	41.8	0.20
1–3 times/week	472	105	22.2	158	33.5	209	44.3	
4+ times/week	268	45	16.8	99	36.9	124	46.3	
Dairy products								
Daily	443	97	21.9	151	34.1	195	44.0	0.92
1–3 times/week	237	50	21.1	83	35.0	104	43.9	
< 2 times/month	158	30	19.0	53	33.5	75	47.5	
Strict vegetarian								
No	785	166	21.1	278	35.4	341	43.4	0.01
Yes	53	11	20.8	9	17.0	33	62.3	

[a] *1 serving = approximately 70 g.*
[b] *1 serving = 1 egg.*

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