Serum folate and vitamin B_{12} status in healthy Iranian adults

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مستوى الفولات وفيتامين B12 في مصل الإيرانيين البالغين الأصحاء

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الخلاصة: لتقييم وضع فولات المصل وفيتامين B12 في مصل الإيرانيين البالغين الأصحاء، صمم الباحثون دراسة عرضية مرتكزة على السكان شملت 1200 فرداً ممن تتراوح أعمارهم بين 20 و80 عاماً. وانتهى بهم المطاف إلى تقييم 984 مشاركاً (507 رجلاً و477 امرأة) ووجدوا أن وسطي مستوى الفولات في المصل 4.61 نانو غرام/ ميلي لتر (بانحراف معياري 2.40) وأن وسطي مستوى الفيتامين B12 يبلغ 265.6 بيكو غرام/ ميلي لتر (بانحراف معياري 170.9)، وكان لدى 1٪ منهم عوز في الفولات، ولدى 25.8٪ منهم مستويات منخفضة لفيتامين B12، وذلك وفقاً للمدى المرجعي الذي حددته الشركة الصانعة (وهو أقل من 1.5 نانو غرام/ ميلي للفولات، وأقل من 160 بيكو غرام لفيتامين B12). كما لوحظ أن المستوى الوسطي للفولات وفيتامين B12 في المصل أخفض بدرجة يعتد بها إحصائياً لدى الرجال، وكان معدَّل انتشار عوز فيتامين B12 أعلى بشكل ملحوظ من عوز الفولات. ويبدو أن من الضروري اتخاذ إجراءات وقائية.

ABSTRACT To assess the serum folate and vitamin B_{12} status in healthy Iranian adults, we designed a population-based cross-sectional study of 1200 individuals aged 20–80 years. Finally 984 participants (507 men and 477 women) were assessed. The mean serum folate was 4.61 (SD 2.40) ng/mL and the mean serum vitamin B_{12} level was 265.6 (SD 170.9) pg/mL. Overall 1.0% were folate deficient and 25.8% had low vitamin B_{12} levels according to the manufacturer's reference ranges (folate < 1.5 ng/mL and vitamin B_{12} < 160 pg/mL). The mean serum folate and vitamin B_{12} levels were significantly lower in men. The prevalence of vitamin B_{12} deficiency was considerably higher than folate deficiency. Implementation of preventive measures seems to be necessary.

Taux sériques de folate et de vitamine B_{12} chez les adultes iraniens en bonne santé

RÉSUMÉ Afin d'évaluer les taux sériques de folate et de vitamine B_{12} chez les adultes iraniens en bonne santé, nous avons conçu une étude transversale en population sur 1 200 sujets âgés de 20 à 80 ans. Finalement, 984 participants (507 hommes et 477 femmes) ont été évalués. Le taux moyen de folate sérique était de 4,61 (E.T. 2,40) ng/ml et celui de vitamine B_{12} sérique, de 265,6 (E.T. 170,9) pg/ml. Globalement, 1,0 % des sujets présentaient une déficience en folate et 25,8 % avaient de faibles taux de vitamine B_{12}, d'après les valeurs de référence du fabricant (folate < 1,5 ng/ml et vitamine B_{12} < 160 pg/ ml). Les taux moyens de folate et de vitamine B_{12} sériques étaient significativement plus bas chez les hommes. La prévalence de la déficience en vitamine B_{12} était considérablement plus élevée que celle de la déficience en folate. La mise en œuvre de mesures préventives semble nécessaire.

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Introduction

Vitamin B_{12} (cobalamin) and/or folic acid deficiency can cause a characteristic megaloblastic anaemia. While deficiency of cobalamin and folic acid produce anaemia, only vitamin B_{12} deficiency has the potential to cause neurological changes [*1–4*]. Therefore accurate identification of vitamin B_{12} deficiency is important because inappropriate treatment with folic acid will correct the haematological signs of vitamin B_{12} deficiency but leave the neurological symptoms unaltered [*5*].

It is generally assumed that due to efficient enterohepatic recycling of the vitamin, B_{12} deficiency is unlikely to occur except in special circumstances such as long-term consumption of a strictly vegetarian diet, pernicious anaemia and malabsorption syndromes. But vitamin B_{12} deficiency is a major worldwide problem, especially in some Asian countries and the Middle East [*6*].

The prevalence of low serum folate concentrations is highly variable. Folate deficiency does not seem to be a serious problem in most populations of the Americas [*7*]. Although folic acid fortification contributed to significant improvement in folate status, serum folate concentrations have declined recently. This may be attributable to lower folic acid intakes [*8*].

There are limited data on serum folate and vitamin B_{12} levels in the Islamic Republic of Iran. In our population the consumption of all types of vegetables is high but meat consumption is lower than Western countries. We hypothesise that in our community, the prevalence of folate deficiency is lower and vitamin B_{12} deficiency is higher than Western communities. We assessed the serum folate and vitamin B_{12} status and age-specific prevalence of folate and vitamin B_{12} deficiency in a population-based study representative of an urban adult Iranian healthy population.

Methods

This was a population-based cross-sectional study with cluster random sampling on healthy individuals in Shiraz carried out between October 2004 and July 2005.

Sample

A total of 1200 (600 men and 600 women) adult Iranian nationals were randomly selected from all regions of Shiraz. All were in the age range 20–80 years. According to the municipality of Shiraz, the city was divided into 88 areas. We selected the areas with odd numbers. In each area, the population between 20 and 80 years of age was selected using a cluster random sampling. The response rate was 95%. Inclusion criteria were being healthy and aged 20–80 years. Those who were vegetarians and those who had any systemic illness, serious organ diseases [renal failure (creatinine $\geq$ 1.4 mg/dL), liver failure], alcoholism, taking food or multivitamin supplements or taking anticonvulsants, antimetabolites and antiviral drugs were excluded. Pregnant and lactating women were also excluded. The local ethics committee approved the study. Informed written consent was obtained from all respondents before being admitted into the study.

Data collection

Participants underwent a standardized medical history, physical examination and anthropometric measurements by an internist. Data were collected using a questionnaire. Blood samples were taken in the morning after a 12-hour overnight fast for serum folate, vitamin B_{12} and creatinine levels. The serum was separated within 1 hour of sampling by centrifugation (20 min, room temperature, at 2000 rpm) and was stored at –70 ºC until analysis.

Folate and vitamin B_{12} levels were measured simultaneously by a radioim-

munoassay kit (SimulTRAC kit, MP Biomedicals, United States). The intra-assay coefficients of variation for folate and vitamin B_{12} were 5.1% and 6.1% respectively. The inter-assay coefficients of variation for folate and vitamin B_{12} were 7.5% and 8.2% respectively. Serum creatinine was measured using commercially available kits on the Cobas autoanalyser.

Folate deficiency was defined as serum folate level < 1.5 ng/mL and vitamin B_{12} deficiency as serum vitamin B_{12} concentration < 160 pg/mL according to the manufacturer's reference range. For comparison with other studies we calculated the prevalence of low serum folate and vitamin B_{12} levels at different cut-off values.

Statistical analysis

Data were analysed using *SPSS*, version 13.0. The mean and standard deviation (SD) of serum folate and vitamin B_{12} levels were compared between sex groups by independent sample *t*-test. Pearson correlation coefficient was used to determine the correlation between age, serum folate and vitamin B_{12} levels. The chi-squared test was used to compare sex groups for prevalence of low serum folate and vitamin B_{12} levels. The Mann–Whitney test was used to compare serum folate and vitamin B_{12} levels between 2 age groups (< 60 and ≥ 60 years). The prevalence of low serum folate and vitamin B_{12} levels was compared between the 2 age groups by the chi-squared test. $P < 0.05$ was considered statistically significant.

Results

Of the 1200 people selected for the study, 216 were excluded; 105 had a history of systemic illness (e.g. diabetes mellitus, liver cirrhosis), 79 were on vitamin and/or supplement therapy; 26 had serum creatinine level ≥ 1.4 mg/dL and 6 were pregnant. Finally, 984 respondents (507 men and 477 women) were assessed.

The mean age was 44.5 (SD 14.7) years. The mean serum folate concentration was 4.61 (SD 2.40) ng/mL and the mean serum vitamin B_{12} level was 265.6 (SD 170.9) pg/mL. There was a significant positive correlation between serum folate level and age of the participants ($r = 0.102$, $P = 0.001$). There was no correlation between serum vitamin B_{12} concentration and age ($r = –0.25$, $P = 0.44$). The serum folate and vitamin B_{12} levels were positively and significantly correlated ($r = 0.65$, $P = 0.0001$) in both sexes (men: $r = 0.745$, $P = 0.0001$; women: $r = 0.622$, $P = 0.0001$). A total of 1.0% of all participants were folate deficient (folate < 1.5 ng/mL) and 25.8% had low vitamin B_{12} level (vitamin B_{12} < 160 pg/mL) according to the manufacturer's reference range. The mean serum folate and vitamin B_{12} levels of all participants and prevalence of folate and vitamin B_{12} deficiency (with different cut-off points) according to age group are shown in Table 1.

The mean serum folate ($t = 7.561$, $P = 0.0001$) and mean vitamin B_{12} ($t = 3.385$, $P = 0.001$) concentrations were significantly lower in men than women. In men, there was no correlation of serum folate ($r = 0.061$, $P = 0.17$) or vitamin B_{12} ($r = 0.052$, $P = 0.24$) levels with age. In women, there was a significant positive correlation between serum folate level and age ($r = 0.136$, $P = 0.003$), while there was a significant negative correlation between vitamin B_{12} level and age ($r = –0.099$, $P = 0.03$). The mean serum folate and vitamin B_{12} levels and prevalence of folate and vitamin B_{12} deficiency (with different cut-off points) in each sex are shown in Tables 2 and 3. The sex groups were also compared for prevalence of low serum folate and vitamin B_{12} levels. The prevalence of folate deficiency was significantly higher in men according

to the serum folate cut-off values of 3.0 and 5.0 ng/mL. The prevalence of low serum vitamin B_{12} was also higher in men according to the serum vitamin B_{12} cut-off points of 200 and 250 pg/mL.

The participants were divided into 2 age groups: < 60 and ≥ 60 years. In 180 of the older age group (84 men and 96 women), there was significant positive correlation between serum vitamin B_{12} level and age ($r = 0.18$, $P = 0.015$), but there was no correlation between serum folate concentration and age ($r = 0.031$, $P = 0.68$). There was also a significant positive correlation between serum folate and vitamin B_{12} level ($r = 0.41$, $P = 0.0001$). The mean serum folate level was significantly lower in older age group men than older women [4.39 (SD 1.9) versus 5.88 (SD 4.3) ng/mL] ($t = 3.0$, $P = 0.003$). But, the mean serum vitamin B_{12} concentration was not significantly different between the sex groups of older participants [men: 278.8 (SD 210.2) pg/mL; women: 254.6 (SD 164.1) pg/mL] ($t = –0.86$, $P = 0.37$). Although serum folate level was significantly higher in the older age group ($z = –2.34$, $P = 0.019$), the serum vitamin B_{12} level showed no significant difference between the 2 age groups ($z = –0.43$, $P = 0.67$).

Table 1 **Mean serum folate and vitamin B_{12} levels and prevalence of deficiency in all participants by age group**

Age (years)	No.	Folate (ng/mL)	Vitamin B_{12} (pg/mL)	Low folate level						Low vitamin B_{12} level					
				< 1.5 ng/mL		< 3.0 ng/mL		< 5.0 ng/mL		< 160 pg/mL		< 200 pg/mL		< 250 pg/mL	
		Mean (SD)	Mean (SD)	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
20–29	199	4.63 (2.3)	301.3 (192.6)	0	0.0	40	20.1	134	67.3	40	20.1	65	32.7	95	47.7
30–39	209	4.23 (1.7)	251.0 (147.1)	4	1.9	37	17.7	153	73.2	57	27.3	91	43.5	125	59.8
40–49	197	4.34 (1.9)	242.7 (132.4)	2	1.0	39	19.8	137	69.5	50	25.4	77	39.1	119	60.4
50–59	199	4.74 (2.5)	267.6 (184.9)	4	2.0	36	18.1	132	66.3	51	25.6	73	36.7	109	54.8
≥ 60	180	5.19 (3.5)	265.9 (186.9)	0	0.0	30	16.7	106	58.8	56	31.1	71	39.4	101	56.1
Total	984	4.61 (2.4)	265.6 (170.9)	10	1.0	182	18.5	662	67.3	254	25.8	377	38.3	549	55.8

Discussion

In this report we present up-to-date data on serum folate and vitamin B_{12} levels in an urban population of the Islamic Republic of Iran. According to the manufacturer's reference range, folate deficiency was seen in 1% and vitamin B_{12} deficiency in about 26% of our population. Also our results showed that the prevalence of folate and vitamin B_{12} deficiency was higher in men, which is consistent with previous reports [*9–11*]. A major problem when comparing the prevalence of low levels of vitamins across studies is the variability of cut-offs used to denote deficiency. Thus, in this study we calculated the prevalence based on different cut-off points used in various studies. The distribution of serum folate and vitamin B_{12} levels has been reported in some populations of other countries but there is little available information describing these parameters in the

Table 2 **Mean serum folate and vitamin B_{12} levels and prevalence of deficiency in men by age group**

Age (years)	No.	Folate (ng/mL)	Vitamin B_{12} (pg/mL)	Low folate level						Low vitamin B_{12} level					
				< 1.5 ng/mL		< 3.0 ng/mL		< 5.0 ng/mL		< 160 pg/mL		< 200 pg/mL		< 250 pg/mL	
		Mean (SD)	Mean (SD)	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
20–29	98	4.08 (1.9)	264.6 (167.3)	0	0.0	25	25.5	79	80.6	22	22.4	38	38.8	58	59.2
30–39	111	3.99 (1.5)	237.8 (144.2)	2	1.8	22	19.8	87	78.4	31	27.9	53	47.8	70	63.1
40–49	106	3.89 (1.4)	224.9 (114.8)	2	1.9	24	22.6	86	81.1	32	30.2	42	39.6	68	64.2
50–59	108	3.96 (1.6)	240.9 (156.6)	4	3.7	26	24.0	84	77.8	31	28.7	46	42.6	66	61.1
≥ 60	84	4.39 (1.9)	278.8 (159.4)	0	0.0	15	17.9	61	72.6	26	30.9	35	41.7	50	59.5
Total	507	4.05 (1.7)	247.7 (159.4)	8	1.6	112	22.1	397	78.3	142	28.0	214	42.2	312	61.5

Table 3 **Mean serum folate and vitamin B_{12} levels and prevalence of deficiency in women by age group**

Age (years)	No.	Folate (ng/mL)	Vitamin B_{12} (pg/mL)	Low folate level						Low vitamin B_{12} level					
				< 1.5 ng/mL		< 3.0 ng/mL		< 5 ng/mL		< 160 pg/mL		< 200 pg/mL		< 250 pg/mL	
		Mean (SD)	Mean (SD)	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
20–29	101	5.16 (2.4)	336.9 (209.0)	0	0.0	15	14.9	55	54.5	18	17.8	27	26.7	37	36.6
30–39	98	4.49 (1.9)	265.9 (149.7)	2	2.0	15	15.3	66	67.3	26	26.5	38	38.8	55	56.1
40–49	91	4.87 (2.2)	263.5 (148.3)	0	0.0	15	16.5	51	56.0	18	19.8	35	38.5	51	56.0
50–59	91	5.65 (3.1)	299.3 (210.1)	0	0.0	10	11.0	48	52.7	20	22.0	27	29.7	43	47.3
≥ 60	96	5.88 (2.9)	254.6 (164.1)	0	0.0	15	15.6	45	46.9	30	31.3	36	37.5	51	53.1
Total	477	5.21 (2.9)	284.6 (180.5)	2	0.4	70	14.7	265	55.6	112	23.5	163	34.2	237	49.7

Table 4 **Number of participants with low serum folate and low vitamin B_{12} levels by sex (*n* = 984)**

Sex	Low folate level			Low vitamin B_{12} level		
	< 1.5 ng/mL	< 3.0 ng/mL	< 5.0 ng/mL	< 160 pg/mL	< 200 pg/mL	< 250 pg/mL
	No.	No.	No.	No.	No.	No.
Male	8	112	397	142	214	312
Female	2	70	265	112	163	237
Total	10	182	662	254	377	549
P-value[a]	0.11	0.003	< 0.001	0.11	0.01	< 0.001

[a]*Chi-squared test.*

Eastern Mediterranean Region, especially in Iranians.

To our knowledge there is only one published study that has reported serum folate and vitamin B_{12} in Iranians, a study in Tehran on 1214 apparently healthy individuals [*12*]. The age-adjusted prevalence of low serum folate level was 98.7% in men and 97.9% in women and the age-adjusted prevalence of low serum vitamin B_{12} level was 26.3% in men and 27.2% in women. A folate concentration < 11 nmol/L (equivalent to 5 ng/mL) and a vitamin B_{12} level < 185 pmol/L (equivalent to 250 pg/mL) was the criterion for defining low vitamin levels [*12*]. In our study, a low level of serum folate (cut-off point of 5 ng/mL) was found in 78.3% of men and 55.6% of women, a lower prevalence. In a study on 216 healthy adult volunteers (aged 19–50 years) from north Jordan, a suboptimal (< 222 pg/mL) serum level of vitamin B_{12} was seen in 48.1% of subjects [*13*]. The prevalence of vitamin B_{12} deficiency was similar to our population. In another study on 470 nonpregnant Lebanese women aged 15–45 years, the reported rates of plasma folate and vitamin B_{12} deficiency were 25.1% and 39.4% respectively [*14*].

In a study in Bangladesh on 1650 adults, the mean plasma folate level was reported as 9.8 (SD 6.2) nmol/L [equivalent to 4.32 (SD 2.9) ng/mL] in men and 12.3 (SD 7.6) nmol/L [5.42 (SD 3.5) ng/mL] in women; 57% of men and 39% of women had a low plasma folate concentration (< 9 nmol/L or ≤ 4 ng/mL). The mean plasma cobalamin level was 281 (SD 115) pmol/L [equivalent to 382.2 (SD 156.4) pg/mL] in men and 256.0 (SD 118) pmol/L [348.2 (SD 160.5) pg/mL] in women; folate was lower, whereas cobalamin was higher among men than among women. The prevalence of cobalamin deficiency (< 151 pmol/L or ≤ 205 pg/mL) was 8% for men and 13% for women [*15*]. The mean serum folate level in both sexes was similar to the results of our study. The prevalence of vitamin B_{12} deficiency was higher in our population. In another study on 548 elderly people (aged ≥ 65 years) of the original Framingham study cohort, the serum folate concentration was < 2.6 ng/mL in 2.9% and < 5.0 ng/mL in 23.5% of the subjects; 53% had cobalamin values < 200 pg/mL and serum concentrations < 350 pg/mL were found in 40.5% of the elderly subjects [*16*]. In the age group ≥ 60 years, our population showed a higher prevalence of folate and vitamin B_{12} deficiency at different cut-off points. A population-based cross-sectional analysis of 3511 people aged 65+ years in the United Kingdom revealed that the prevalence of both folate and vitamin B_{12} deficiency increased with age [*17*]. Other studies focusing on elderly people have suggested a prevalence of cobalamin deficiency of around 30%–40% [*18*].

In agreement with several other studies, including the Third National Health and Nutrition Examination Survey (NHANES III), men were found to have lower serum folate concentrations than women [*19,20*], but the reasons for this difference are unclear. We can attribute the difference in the frequency of low serum folate and vitamin B_{12} levels reported by different studies to different cut-off values of serum folate and vitamin B_{12} in defining deficiency, the method used to measure serum folate and vitamin B_{12} levels, dietary habits and geographical distribution or ethnicity of the populations.

Our study has several strengths. First, it was a population-based study with random sampling from all regions of Shiraz, indicating that these findings can be applied to the Iranian urban general population. Secondly, the data on prevalence of low serum folate and vitamin B_{12} levels were presented in different cut-off values for comparison with other studies. Thirdly, we assessed the subjects who had fasted ≥ 12 hours because folate concentration measured in a fasted state is a better indicator of folate status. However, our study has some limitations. The other variables that indicate tissue deficiency of folate and vitamin B_{12}, such as methylmalonic acid (MMA), total homocysteine (tHcy) and red blood cell folate level were not measured. It is better to measure those variables, although the MMA assay is expensive and there is no consensus on the cut-offs of tHcy or MMA to use to define vitamin B_{12} deficiency, especially in elderly populations in which impaired renal function can be an important confounding factor [*10,21–23*].

In conclusion, although some data have been published on the distribution of serum folate and vitamin B_{12} concentrations in our community, this study in a sample of healthy adult Iranians focuses attention on the levels of serum folate and vitamin B_{12} as a potential health problem in the community. Overall, the prevalence of folate and vitamin B_{12} deficiency is high. This may have an impact on the health of our people. We require further investigations for the definitive diagnoses of true folate and vitamin B_{12} deficiency. We recommend that regular use of green leafy vegetables and folic acid fortification of foods be encouraged and, at least in elderly people, generous doses of vitamin B_{12} be given to prevent inappropriate mistreatment of cobalamin deficiency with folic acid.

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