Serum folate and vitamin B\textsubscript{12} status in healthy Iranian adults

M. Shams,\textsuperscript{1} K. Homayouni\textsuperscript{2} and G.R. Omrani\textsuperscript{1}

ABSTRACT To assess the serum folate and vitamin B\textsubscript{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\textsubscript{12} level was 265.6 (SD 170.9) pg/mL. Overall 1.0% were folate deficient and 25.8% had low vitamin B\textsubscript{12} levels according to the manufacturer's reference ranges (folate < 1.5 ng/mL and vitamin B\textsubscript{12} < 160 pg/mL). The mean serum folate and vitamin B\textsubscript{12} levels were significantly lower in men. The prevalence of vitamin B\textsubscript{12} deficiency was considerably higher than folate deficiency. Implementation of preventive measures seems to be necessary.

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

RÉSUMÉ Afin d’évaluer les taux sériques de folate et de vitamine B\textsubscript{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\textsubscript{12} sérique, de 265,6 (E.T. 170,9) pg/ml. Overall 1.0% were folate deficient and 25.8% had low vitamin B\textsubscript{12} levels according to the manufacturer’s reference ranges (folate < 1.5 ng/mL and vitamin B\textsubscript{12} < 160 pg/mL). The mean serum folate and vitamin B\textsubscript{12} levels were significantly lower in men. The prevalence of vitamin B\textsubscript{12} deficiency was considerably higher than folate deficiency. Implementation of preventive measures seems to be necessary.

\textsuperscript{1}Endocrine and Metabolism Research Centre, Namazi Hospital; \textsuperscript{2}Al-Zahra Hospital, Shiraz University of Medical Sciences, Shiraz, Islamic Republic of Iran (Correspondence to G.R. Omrani: hormone@sums.ac.ir). Received: 11/02/07; accepted: 24/04/07
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 Bio-
medicals, United States). The intra-assay
coefficients of variation for folate and vita-
mB12 were 5.1% and 6.1% respectively. The
inter-assay coefficients of variation for folate and vitamin B12 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 B12 de-
ficiency as serum vitamin B12 concentration
< 160 pg/mL according to the manufactur-
er’s reference range. For comparison with
other studies we calculated the prevalence
of low serum folate and vitamin B12 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 B12 levels were
compared between sex groups by independ-
ent sample t-test. Pearson correlation co-
efficient was used to determine the correlation
between age, serum folate and vitamin B12
levels. The chi-squared test was used to
compare sex groups for prevalence of low
serum folate and vitamin B12 levels with
different cut-off points according to age group.
The mean serum folate and vitamin B12
clearance of all participants and prevalence
of folate and vitamin B12 deficiency (with dif-
f erent 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 B12 (t = 3.385,
P = 0.001) concentrations were signifi-
cantly lower in men than women. In men,
there was no correlation of serum folate (r
= 0.061, P = 0.17) or vitamin B12 (r = 0.052,
P = 0.24) levels with age. In women, there
was a significant positive correlation be-
tween serum folate level and age (r = 0.136,
P = 0.003), while there was a significant
negative correlation between vitamin B12
level and age (r = −0.099, P = 0.03). The
mean serum folate and vitamin B12 levels
and prevalence of folate and vitamin B12
deficiency (with different cut-off points) in
each sex are shown in Tables 2 and 3. The
sex groups were also compared for preva-
ience of low serum folate and vitamin B12
deficiency. The prevalence of folate deficien-
cy 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\textsubscript{12} was also higher in men according to the serum vitamin B\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{12} level showed no significant difference between the 2 age groups ($z = -0.43, P = 0.67$).

**Discussion**

In this report we present up-to-date data on serum folate and vitamin B\textsubscript{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\textsubscript{12} deficiency in about 26% of our population. Also our results showed that the prevalence of folate and vitamin B\textsubscript{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-offs used in various studies. The distribution of serum folate and vitamin B\textsubscript{12} levels has been reported in some populations of other countries but there is little available information describing these parameters in the

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No.</th>
<th>Mean (SD)</th>
<th>%</th>
<th>No.</th>
<th>Mean (SD)</th>
<th>%</th>
<th>No.</th>
<th>Mean (SD)</th>
<th>%</th>
<th>No.</th>
<th>Mean (SD)</th>
<th>%</th>
<th>No.</th>
<th>Mean (SD)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>20–29</td>
<td>199</td>
<td>4.63 (2.3)</td>
<td>40</td>
<td>201.1</td>
<td>134</td>
<td>67.3</td>
<td>40</td>
<td>201.1</td>
<td>134</td>
<td>67.3</td>
<td>40</td>
<td>201.1</td>
<td>134</td>
<td>67.3</td>
<td></td>
</tr>
<tr>
<td>30–39</td>
<td>209</td>
<td>4.23 (1.7)</td>
<td>67</td>
<td>197</td>
<td>137</td>
<td>73.2</td>
<td>67</td>
<td>197</td>
<td>137</td>
<td>73.2</td>
<td>67</td>
<td>197</td>
<td>137</td>
<td>73.2</td>
<td></td>
</tr>
<tr>
<td>40–49</td>
<td>197</td>
<td>4.34 (1.9)</td>
<td>57</td>
<td>177</td>
<td>113</td>
<td>69.5</td>
<td>57</td>
<td>177</td>
<td>113</td>
<td>69.5</td>
<td>57</td>
<td>177</td>
<td>113</td>
<td>69.5</td>
<td></td>
</tr>
<tr>
<td>50–59</td>
<td>189</td>
<td>4.74 (2.5)</td>
<td>50</td>
<td>254</td>
<td>181</td>
<td>106.3</td>
<td>50</td>
<td>254</td>
<td>181</td>
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<td>50</td>
<td>254</td>
<td>181</td>
<td>106.3</td>
<td></td>
</tr>
<tr>
<td>≥ 60</td>
<td>180</td>
<td>5.19 (3.5)</td>
<td>56</td>
<td>31.1</td>
<td>31.1</td>
<td>31.1</td>
<td>56</td>
<td>31.1</td>
<td>31.1</td>
<td>31.1</td>
<td>56</td>
<td>31.1</td>
<td>31.1</td>
<td>31.1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>984</td>
<td>4.61 (2.4)</td>
<td>254</td>
<td>37.7</td>
<td>37.7</td>
<td>37.7</td>
<td>254</td>
<td>37.7</td>
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<td>254</td>
<td>37.7</td>
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</tr>
</tbody>
</table>
### Table 2 Mean serum folate and vitamin B<sub>12</sub> levels and prevalence of deficiency in men by age group

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

### Table 3 Mean serum folate and vitamin B<sub>12</sub> levels and prevalence of deficiency in women by age group

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No.</th>
<th>Folate (ng/mL) Mean (SD)</th>
<th>Vitamin B&lt;sub&gt;12&lt;/sub&gt; (pg/mL) Mean (SD)</th>
<th>Low folate level &lt; 1.5 ng/mL No. %</th>
<th>&lt; 3.0 ng/mL No. %</th>
<th>&lt; 5.0 ng/mL No. %</th>
<th>&lt; 160 pg/mL No. %</th>
<th>&lt; 200 pg/mL No. %</th>
<th>&lt; 250 pg/mL No. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>20–29</td>
<td>101</td>
<td>5.16 (2.4)</td>
<td>336.9 (209.0)</td>
<td>0  0.0</td>
<td>15  14.9</td>
<td>55  54.5</td>
<td>18  17.8</td>
<td>27  26.7</td>
<td>37  36.6</td>
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<tr>
<td>30–39</td>
<td>98</td>
<td>4.49 (1.9)</td>
<td>265.9 (149.7)</td>
<td>2  2.0</td>
<td>15  15.3</td>
<td>66  67.3</td>
<td>26  26.5</td>
<td>38  38.8</td>
<td>55  56.1</td>
</tr>
<tr>
<td>40–49</td>
<td>91</td>
<td>4.87 (2.2)</td>
<td>263.5 (148.3)</td>
<td>0  0.0</td>
<td>15  16.5</td>
<td>51  56.0</td>
<td>18  19.8</td>
<td>35  38.5</td>
<td>51  56.0</td>
</tr>
<tr>
<td>50–59</td>
<td>91</td>
<td>5.65 (3.1)</td>
<td>299.3 (210.1)</td>
<td>0  0.0</td>
<td>10  11.0</td>
<td>48  52.7</td>
<td>20  22.0</td>
<td>27  29.7</td>
<td>43  47.3</td>
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<tr>
<td>≥ 60</td>
<td>96</td>
<td>5.88 (2.9)</td>
<td>254.6 (164.1)</td>
<td>0  0.0</td>
<td>15  15.6</td>
<td>45  46.9</td>
<td>30  31.3</td>
<td>36  37.5</td>
<td>51  53.1</td>
</tr>
<tr>
<td>Total</td>
<td>477</td>
<td>5.21 (2.9)</td>
<td>284.6 (180.5)</td>
<td>2  0.4</td>
<td>70  14.7</td>
<td>265 55.6</td>
<td>112 23.5</td>
<td>163 34.2</td>
<td>237 49.7</td>
</tr>
</tbody>
</table>
Eastern Mediterranean Region, especially in Iranians.

To our knowledge there is only one published study that has reported serum folate and vitamin B\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{12} was seen in 48.1% of subjects [13]. The prevalence of vitamin B\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{12} deficiency increased with age [17]. Other studies focusing on elderly people have suggested a prevalence of cobalamin deficiency of around 30%–40% [18].

### Table 4

<table>
<thead>
<tr>
<th>Sex</th>
<th>Low folate level</th>
<th>Low vitamin B\textsubscript{12} level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>No.</td>
</tr>
<tr>
<td>Male</td>
<td>8</td>
<td>112</td>
</tr>
<tr>
<td>Female</td>
<td>2</td>
<td>70</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>182</td>
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<tr>
<td>P-value*</td>
<td>0.11</td>
<td>0.003</td>
</tr>
</tbody>
</table>

*Chi-squared test.
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 \(\geq 12\) 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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References


