

Folate deficiency is associated with nutritional anaemia in Lebanese women of childbearing age

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Abstract

Objective: The objective of this study was to identify the determinants of anaemia in Lebanese women of childbearing age attending health centres in Lebanon.

Design: Cross-sectional study carried out between May and December 2003. Anthropometric measurements as well as sociodemographic, health and dietary intake data were collected using a questionnaire. Haemoglobin (Hb), plasma ferritin, plasma folate and vitamin B₁₂ were assessed using standard laboratory methods.

Setting: Governmental health centres in Lebanon.

Subjects: Four hundred and seventy non-pregnant Lebanese women aged 15–45 years.

Results: Anaemia (Hb <12 g dl⁻¹) and iron deficiency (ferritin <15 µg l⁻¹) were prevalent in 16.0 and 27.2% of the study sample, respectively. Of the total sample, 7.7% had iron-deficiency anaemia. The percentage of women with either Hb or ferritin deficiency or both was 35.6%. Plasma folate and vitamin B₁₂ deficiency was reported in 25.1 and 39.4%, respectively, and 12.6% of the women had both folate and vitamin B₁₂ deficiencies. Of the anaemic group, 48.0% of the women had iron deficiency. The intake of iron was lower in iron-deficient than in non-deficient women and a positive relationship was shown between folate intake and its corresponding serum levels. Regression analysis showed that ferritin, plasma folate and family history of anaemia were significant determinants of the anaemia in the sample of women.

Conclusions: Anaemia not related to iron deficiency was partly explained by plasma folate deficiency. Measures to control folate and iron deficiency should be considered.

Keywords

Anaemia
Iron
Ferritin
Folate

Women of childbearing age
Lebanon

Anaemia remains a major global public health problem, affecting about a quarter of the world's population. Its adverse health consequences affect people with varied degrees of affluence and from all age groups, particularly women of childbearing age and children. The micronutrient status of high-risk populations has recently received extensive attention as it adversely affects pregnancy outcome, working ability, intellectual capacity and immunity¹. The World Health Organization (WHO) estimates that 42.3% of women in non-industrialised countries suffer from anaemia versus only 10.3% in industrialised ones². International efforts attempting to eradicate anaemia have so far failed. Anaemia can result from non-nutritional factors such as haemorrhage, infection, chronic disease states or drug toxicity, as well as from nutritional ones including deficiencies of iron, specific vitamins, copper and protein. Iron deficiency remains the major cause of anaemia and is the most

widespread single nutrient deficiency in the world. It is estimated that 75% of anaemia is due to iron deficiency, followed by folate and vitamin B₁₂ deficiencies³.

Several studies indicate that nutritional anaemia in women of childbearing age may result from, in addition to iron, other micronutrient deficiencies such as folate and vitamin B₁₂^{4–10}. Folate and B₁₂ deficiency can lead to anaemia mainly through their role in thymidylate and DNA synthesis, thus leading to ineffective erythropoiesis^{4,11}. Identifying the magnitude of anaemia and its determinants in women is essential for evidence-based intervention modalities, particularly in developing countries where awareness is lacking and resources are scarce^{9,12,13}.

In Lebanon, a small middle-income country in the Mediterranean region, micronutrient deficiencies have been suspected to constitute a public health problem. In an earlier study conducted in a representative sample of Lebanese women of childbearing age in 1998,

haemoglobin deficiency ($<12 \text{ g dl}^{-1}$) was estimated at 21.4% and low plasma ferritin ($<15 \mu\text{g l}^{-1}$) was present in 33.6% of the women surveyed¹⁴. Of the anaemic group 37.4% were not iron-deficient. Other determinants of anaemia were not investigated in that study. Given the above, the present study investigated, in addition to iron, other determinants of nutritional anaemia in a sample of Lebanese women of childbearing age.

Methods

Study design and sample

The study sample was drawn from women of childbearing age (15–45 years) attending health centres across Lebanon. The list of health centres was provided by the Ministry of Social Affairs in Lebanon; these centres are generally attended by families of low to moderate socioeconomic status. A random sample of 23 health centres was drawn proportionate to their distribution in the six districts (Muhafazats) across Lebanon¹⁵. A total of 22 subjects drawn randomly from each centre and proportionate to their age distribution in the districts (grand total = 506) were invited to participate in the study. Pregnant and lactating women were excluded from the sample. All women signed an informed consent form before being enrolled in the study. Refusals did not exceed 2% of the sample and 93% of the women (470) completed a questionnaire and provided a blood sample. The Institutional Review Board of the American University of Beirut approved the study protocol and fieldwork was carried out between May and December 2003.

Data collection procedures

The questionnaire covered information on sociodemographic characteristics, a brief medical history and family history of anaemia. Dietary intake was assessed using 24-hour dietary recall. Height was measured without shoes and recorded to the nearest 0.5 cm; weight was measured in light indoor clothing to the nearest 0.1 kg using a calibrated scale. A 10-ml fasting venous blood sample was drawn from each subject, by a registered nurse, into a sterile tube containing ethylenediaminetetraacetic acid. Blood samples were immediately placed in an icebox for a period not exceeding 3 h. Blood samples were centrifuged and frozen for later analysis.

Blood and laboratory measurements

Hb concentration was estimated on-site using a HemoCue haemoglobinometer (HemoCue AB, Angelholm, Sweden) which was checked on a daily basis using the control cuvette supplied with the photometer. Plasma ferritin was determined using the N Latex Ferritin Kit (Dade Behring Marburg GmbH, Marburg, Germany). Plasma folate and vitamin B₁₂ were analysed using the Abbott AXSYM System (Abbott Laboratories, Abbott Park, IL, USA).

Biochemical analyses were carried out at the Medical Center of the American University of Beirut.

Definition of anaemia, iron deficiency and iron-deficiency anaemia

Following WHO and international guidelines², anaemia was defined as Hb $<12 \text{ g dl}^{-1}$, iron deficiency as ferritin $<15 \mu\text{g l}^{-1}$, and iron-deficiency anaemia (IDA) as Hb $<12 \text{ g dl}^{-1}$ and ferritin $<15 \mu\text{g l}^{-1}$. Plasma folate and vitamin B₁₂ deficiencies were defined as plasma levels $<6.6 \text{ ng ml}^{-1}$ and $<300 \text{ pg ml}^{-1}$, respectively. The cut-off values for the various risk deficiencies (low, moderate and high) were adopted^{2,9,16}.

Statistical analysis

Descriptive statistics were calculated and results are expressed as mean values, standard deviations (SD) and proportions. Statistics used included Student's *t*-test and the chi-square test for continuous and categorical variables, respectively. Dietary consumption of macro- and micronutrients was analysed using Nutritionist Pro™ software (First Databank Inc., San Bruno, CA, USA). The Middle East food composition tables were used to analyse local dishes¹⁷. Multivariate logistic regression analysis was carried out with Hb deficiency ($<12 \text{ g dl}^{-1}$) as the dependent variable and baseline characteristics as covariates. Prevalence odds ratios (OR) and their 95% confidence intervals (CI) were calculated. A *P*-value <0.05 was considered significant. The Statistical Package for the Social Sciences version 12.0 (SPSS for Windows; SPSS Inc., Chicago, IL, USA) was used for all computations.

Results

The final sample consisted of 470 Lebanese women with a mean age of 28.6 (SD 8.7) years. The majority (67.2%) had attained intermediate education comparable to 10 years of schooling and 20.6% reported a family history of anaemia.

Biochemical findings

Haemoglobin and ferritin

Table 1 presents the biochemical indices of the total sample of women and shows their distribution by risk category. Mean Hb and ferritin values were 13.2 g dl^{-1} and $34.1 \mu\text{g l}^{-1}$, respectively. Anaemia (Hb $<12 \text{ g dl}^{-1}$) was present in 16.0% of the women, of whom the majority were at low risk, and iron deficiency (ferritin $<15 \mu\text{g l}^{-1}$) in 27.2%.

The study sample was further classified according to both Hb concentration and plasma ferritin value (Table 2). Sixty-four per cent of the women had normal Hb and ferritin values and 7.7% suffered from IDA. The findings also showed that 48% of the anaemic women had iron deficiency.

Table 1 Distribution of biochemical indices (haemoglobin, plasma ferritin, plasma folate and plasma vitamin B₁₂) of Lebanese women of childbearing age by risk category*

	At-risk groups				Total
	High	Moderate	Low	Subtotal	
Haemoglobin					13.2 ± 1.4
Cut-off (g dl ⁻¹)	< 7.0	7.0–9.9	10–11.9	< 12.0	
Sample	1 (0.2)	11 (2.3)	63 (13.4)	75 (16.0)	
Ferritin					34.1 ± 30.9
Cut-off (µg ml ⁻¹)			< 15.0	< 15.0	
Sample			128 (27.2)	128 (27.2)	
Folate					8.4 ± 2.7
Cut-off (ng ml ⁻¹)	≤ 3.0	3.1–6.0	6.1–6.5	< 6.6	
Sample	1 (0.2)	75 (16.0)	42 (8.9)	118 (25.1)	
Vitamin B ₁₂					378.7 ± 187.5
Cut-off (pg ml ⁻¹)	< 150	150–240	240–300	< 300	
Sample	8 (1.7)	88 (18.7)	89 (18.9)	185 (39.4)	

Data are expressed as *n* (%) or mean ± standard deviation.* Cut-off values for the various risk deficiencies were adopted^{2,9,16}.**Table 2** Classification of Lebanese women of childbearing age (*n* = 470) by risk groups for anaemia and iron deficiency

Haematological status	<i>n</i>	%	Hb (g dl ⁻¹)	PF (µg l ⁻¹)
Normal	303	64.5	13.6 ± 0.9	44.0 ± 32.0
Iron deficiency only (PF < 15 µg l ⁻¹)	92	19.6	13.5 ± 1.0	9.0 ± 3.4
Anaemia only (Hb < 12 g dl ⁻¹)	39	8.3	11.1 ± 0.9	41.4 ± 23.4
Iron-deficiency anaemia (Hb < 12 g dl ⁻¹ and PF < 15 µg l ⁻¹)	36	7.7	10.8 ± 1.2	7.6 ± 4.4

Hb – haemoglobin; PF – plasma ferritin.

Data are expressed as mean ± standard deviation unless indicated otherwise.

Folate and vitamin B₁₂

Mean plasma folate and vitamin B₁₂ levels were 8.4 ng ml⁻¹ and 378.7 pg ml⁻¹, respectively. Folate deficiency was prevalent in 25.1% of the women, with the majority being at moderate risk. Vitamin B₁₂ deficiency was prevalent in 39.4% of the women, with the majority being at low and moderate risk (Table 1). Furthermore, 12.6% of the women had both folate and vitamin B₁₂ deficiencies (Table 3). The percentages of woman with folate deficiency and with both folate and vitamin B₁₂ deficiencies were significantly higher in the iron-deficient anaemic group than in the iron-deficient non-anaemic group (41.7% vs. 22.8% and 30.6% vs. 7.6%, respectively; Table 3).

Food intake data

The mean intake, mean intake as a percentage of the recommended dietary allowance (RDA) and the percentage of women consuming less than 2/3 of the RDA for energy and anaemia-relevant micronutrients are shown in Table 4. In general, the mean energy intake of the women was below the RDA for their age, reaching 71.9%. Consumption of fat reached 39.4% of the total energy intake. Analysis of micronutrient intakes showed that the diets of the women attending health centres in Lebanon were inadequate in iron, folate and vitamin B₁₂.

Analysis of the 24-hour recall data revealed that the majority of the women's dietary iron came from plant

sources of low iron bioavailability such as cereals, beans and vegetables, rather than from animal iron sources, such as meat and dairy products (data not shown). Furthermore, vegetables were the major source of folate in the diet followed by beans and then bread and cereals. Meat, poultry and fish groups were the highest contributors to vitamin B₁₂ in the diet.

The mean iron intake was higher in women with normal ferritin (13.5 ± 10.3 mg) than in women with ferritin deficiency (12.8 ± 6.9 mg). A significant positive correlation was observed between folate intake and its corresponding plasma levels. Women who had plasma folate deficiency had a lower mean folate intake (248.7 ± 220.5 µg) than those with normal serum folate levels (284.9 ± 214.3 µg).

Logistic regression

The results of logistic regression analysis, carried out with Hb deficiency (< 12 g dl⁻¹) as the dependent variable, are presented in Table 5. Ferritin, folate and family history of anaemia were the only variables that yielded a significant result (*P* < 0.05).

Discussion

The present study examined the determinants of anaemia, including – in addition to Hb and ferritin – folate

Table 3 Prevalence of folate and vitamin B₁₂ deficiencies among Lebanese women of childbearing age according to anaemia (Hb < 12 g dl⁻¹) and iron deficiency (PF < 15 µg l⁻¹) status

	Anaemic women (n = 75)		Non-anaemic women (n = 395)		Total (n = 470)
	Iron deficient (n = 36)	Not iron deficient (n = 39)	Iron deficient (n = 92)	Not iron deficient (n = 303)	
Folate deficiency only	4 (11.1)	5 (12.8)	14 (15.2)	36 (11.9)	59 (12.6)
Vitamin B ₁₂ deficiency only	6 (16.7)	11 (28.2)	28 (30.4)	81 (26.7)	126 (26.8)
Both folate and vitamin B ₁₂ deficiency	11 (30.6)	5 (12.8)	7 (7.6)	36 (11.9)	59 (12.6)
None	15 (41.7)	18 (46.2)	43 (46.7)	150 (49.5)	226 (48.0)

Hb – haemoglobin; PF – plasma ferritin.

Data are expressed as n (%).

Table 4 Mean intake, mean intake as a percentage of the RDA and percentage of women consuming less than 2/3 of the RDA for energy and selected nutrients

Nutrient	Intake	Intake as % of RDA	% consuming < 2/3 RDA
Energy (kcal)	1726.6 ± 662.0	71.9 ± 27.5	43.0
Total iron (mg)	13.2 ± 9.4	73.4 ± 52.1	56.2
Folate (µg)	275.8 ± 216.2	68.95 ± 54.1	59.7
Vitamin B ₁₂ (µg)	3.2 ± 6.1	134.5 ± 252.6	41.5

RDA – recommended dietary allowance.

Data are expressed as mean ± standard deviation unless indicated otherwise.

and vitamin B₁₂, in Lebanese women of childbearing age. Based on the Hb results, and consistent with WHO public health classification guidelines, anaemia in Lebanese women could be classified as of mild prevalence². Sixteen per cent of the total sample was anaemic and 27.2% were iron-deficient. Almost 50% of anaemia was related to iron deficiency. This is in agreement with many studies showing that the contribution of iron deficiency to anaemia lies in the range of 40–60% except in children,

Table 5 Associations of anaemia (Hb < 12 g dl⁻¹) with baseline covariates† in the total sample of women (n = 470)

Variable (reference category)	OR (95% CI)
Plasma ferritin	
Normal (≥ 15 µg l ⁻¹)	1.0
Deficient (< 15 µg l ⁻¹)	3.2* (1.9–5.5)
Plasma folate	
Normal (≥ 6.6 ng ml ⁻¹)	1.0
Deficient (< 6.6 ng ml ⁻¹)	1.9* (1.1–3.5)
Plasma vitamin B ₁₂	
Normal (> 300 pg ml ⁻¹)	1.0
Deficient (≤ 300 pg ml ⁻¹)	1.1 (0.6–1.9)
Food intake of vitamin B ₁₂	
< 2/3 RDA	1.0
≥ 2/3 RDA	1.0 (0.6–1.7)
Family history of anaemia	
No	1.0
Yes	3.1* (1.8–5.5)
BMI	
Underweight and normal (< 25 kg m ⁻²)	1.0
Overweight and obese (≥ 25 kg m ⁻²)	1.4 (0.8–2.3)

Hb – haemoglobin; OR – odds ratio; CI – confidence interval; RDA – recommended dietary allowance; BMI – body mass index.

* P < 0.05.

† Controlling for education.

where anaemia is predominantly due to iron deficiency¹¹. The present results also show a lower prevalence of anaemia than reported in a previous study¹⁴, possibly due to improved health care or to differences in sampling procedures, as the present study was conducted on women attending health centres. Socio-economic variables, which included education level, employment status, number of rooms in the household, number of household residents and others, were not significantly associated with anaemia. This agrees with previous observations that anaemia cuts across different sectors of the population including high and low socio-economic groups. Compared to other developing and developed countries (Fig. 1), Lebanese women have a lower prevalence of anaemia than women in India¹⁸, Mexico¹³, Kuwait¹⁹ and Egypt²⁰; however, this level of anaemia is still higher than that in the USA and Europe^{21–23}. Lebanese women of childbearing age have a lower prevalence of ferritin deficiency than women of Jordan²⁴ and Mexico¹³. On the other hand, ferritin deficiency in Lebanon is higher than in Turkey²⁵, Saudi Arabia²⁶, Spain²⁷, New Zealand²⁸ and Denmark²⁹.

It should be noted that although ferritin deficiency was present in 27.2% of the women this number could be an underestimation, since markers of infection were not measured in the present study. It is documented that ferritin is an acute-phase protein and its level rises in cases of infection, thus leading to underestimation of iron deficiency¹¹.

The present study also examined folate and vitamin B₁₂ as other determinants of anaemia. Thirty-nine of the women had anaemia but not iron deficiency. Of these, 12.8% had folate deficiency only and another 12.8% had both folate and vitamin B₁₂ deficiencies. Plasma folate was a significant determinant of Hb status (OR = 1.9, 95% CI 1.1–3.5) and approximately 25.6% of the anaemia experienced by Lebanese women could be explained by folate deficiency. However, the high prevalence of vitamin B₁₂ deficiency observed in our study was not shown to be significantly correlated to anaemia. This may be due to the fact that 95.6% of the vitamin B₁₂-deficient women had moderate to low deficiency, while only 4.3% of the women had severe clinical deficiency. When analysis was done

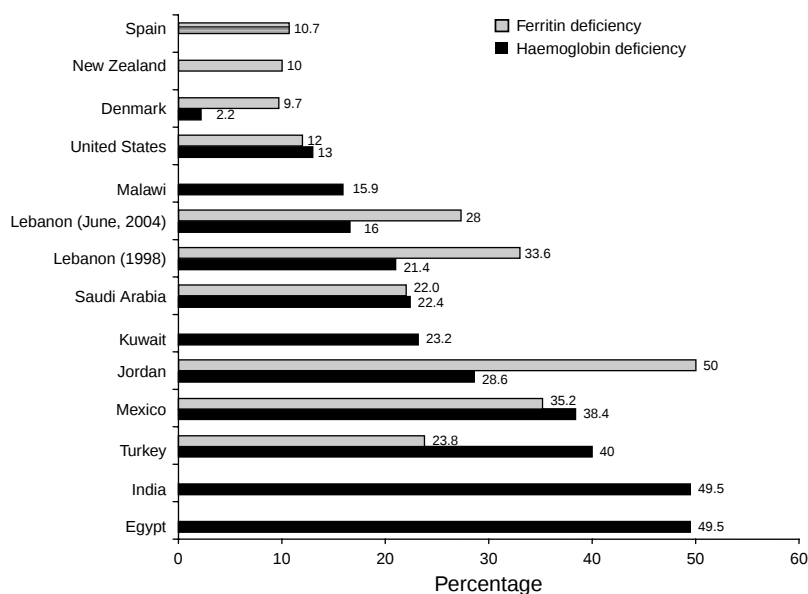


Fig. 1 Haemoglobin and ferritin deficiencies in Lebanese women of childbearing age in comparison to other developed and developing countries^{19–27}

using the cut-off value^{30,31} of 211 pg ml^{-1} , vitamin B₁₂ deficiency was prevalent in 11.5% of the sample of women.

When deficiencies were combined, the percentage of women who had Hb or ferritin or folate or vitamin B₁₂ deficiency was 68.1%. Thus, more than two thirds of the Lebanese women of childbearing age are either anaemic or suffer from deficiency of a major micronutrient. This raises an important public health concern, particularly that deficiency of either iron/folate or vitamin B₁₂ puts the woman and her foetus at risk of perinatal complications, spontaneous miscarriage, intrauterine growth retardation, premature delivery, stunted growth, low birth weight, neural tube defects and pre-eclampsia^{3,6,8,11,32}.

Comparing food intake data with the plasma levels of selected nutrients showed that more than 50% of the women had inadequate intake of iron and folate, and plasma folate was significantly and directly correlated with folate intake. Moreover, the median (221.4 mg) folate intake was approximately equal to 50% of the estimated average requirement for folate³³. Plasma folate is an indicator of recent folate intake and the food intake data were collected through a 24-hour recall, representing recent intake as well. Furthermore, although it is not enriched with folate, bread was a good source of folate in the diets of the women studied as its consumption is very high in Lebanon, especially among people of low to moderate socio-economic status. Plasma B₁₂ was not significantly correlated with B₁₂ intake. This is in line with recent data³⁴ showing that vitamin B₁₂ deficiency may be due to, in addition to insufficient dietary intake, food cobalamin malabsorption syndrome (>60% of all cases) or pernicious anaemia (15–20% of all cases), and malabsorption. Food cobalamin malabsorption has only

recently been identified as a significant cause of cobalamin deficiency among people, and is characterised by the inability to release cobalamin from food or a deficiency of intestinal cobalamin transport proteins or both.

The presence of a family history of anaemia was a strong determinant of Hb status (OR = 3.1, 95% CI 1.8–5.5). It is less likely that disorders such as sickle-cell anaemia and β -thalassaemia are strong contribution factors to anaemia in this population. The frequency of β -thalassaemia in Lebanon is among the lowest in the Mediterranean, being 0.5%³⁵, and the prevalence of sickle-cell disease is less than 1%³⁶.

Conclusion and recommendations

The present study described the status of Hb and ferritin, folate and vitamin B₁₂ deficiencies in Lebanese women of childbearing age. According to the WHO classification of anaemia (Hb < 12 g dl^{-1}), the Lebanese women of childbearing age fall in the category of mild anaemia. In this study, anaemia (Hb < 12 g dl^{-1}) not related to iron deficiency (ferritin < $15 \text{ } \mu\text{g l}^{-1}$) was mostly explained by folate deficiency. Ferritin deficiency and family history of anaemia were the strongest determinants of Hb deficiency in the studied women. Combating iron deficiency in Lebanon is very hard, owing to lack of resources. Since bread was a significant source of folate in the diet of the women even though it is not fortified, the fortification of pita bread with iron and folate, as well as promoting the intake of green leafy vegetables, would help increase the mean iron and folate intakes in these women. More research should be carried out to confirm the magnitude of vitamin B₁₂ deficiency via the assessment of methylmalonic acid, homocysteine, intrinsic factors and

holotranscobalamin receptors¹⁶. Additionally, assessment of C-reactive protein would also rule out the presence of infection and thus validate the actual level of ferritin deficiency in the studied women. Furthermore, the high prevalence of folate deficiency in these women should encourage researchers to investigate the prevalence of neural tube defects in Lebanon.

Limitations

Methylmalonic acid, a good indicator of vitamin B₁₂ deficiency, was not assessed in this study; this is a limitation since we could thus be underestimating the actual extent of vitamin B₁₂ deficiency in the women³⁷. The dietary data in this study were derived from a single 24-hour recall, which is a limitation for precise assessment of food intake.

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