Folate deficiency in women of reproductive age in nine administrative regions of Ethiopia: an emerging public health problem

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Abstract

Objective: To investigate the country-wide extent of folate deficiency and risk factors in Ethiopian women.

Design: Cross-sectional study.

Methods: Multistage cluster sampling and systematic sampling were used to select 970 women aged 15 to 49 years from nine accessible regions of Ethiopia. Demographic and health information was collected via questionnaire. Biological samples were collected by medical technologists. Outcome measures: demographic and health variables, food frequency, haemoglobin status, ferritin status and folate status.

Results: Mean ± SD plasma folate was 5.57 ± 3.84 ng/mL. Forty-six per cent of women had severe folate deficiency (≤ 4 ng/mL) and 21.2% had marginal folate deficiency (> 4–6.6 ng/mL) with unequal prevalence across the country. Severe folate deficiency was higher in women who were unmarried ($p = 0.002$), had parity of 4–6 ($p = 0.001$), used oral contraceptives ($p = 0.01$), had no illnesses ($p = 0.001$), had intestinal parasites (0.001), followed lower plant food diets (0.001), followed lower animal product diets (0.001), had no anaemia (0.001) and had no iron deficiency (0.001). In logistic regression analysis, only low plant food diets ($p = 0.001$) and iron deficiency ($p < 0.001$) retained their significance with regard to folate deficiency. The odds for developing folate deficiency was 0.9 times less likely among those with higher plant food intake (AOR-0.9;95%CI – 0.72–1.2) and 0.2 times less likely among those with adequate iron (AOR-0.2;95%CI – 0.17–0.34).

Conclusions: Folate deficiency is widespread in Ethiopian women, emphasising the need for sustainable folate intake through dietary diversification and appropriate public health measures.

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Introduction

Folate deficiency is a serious problem that affects women worldwide.[1–3] This deficiency is caused primarily by inadequate dietary intake.[3] Typical folate intakes are suboptimal in the diets of many women of childbearing age, and folate intake is further limited by cooking losses and poor bioavailablity,[4] estimated to be from 50% to 82%.[5,6] Fortification of grains with folic acid has increased folate intake in several developed countries,[7] but these foods are generally not available in Ethiopia. Folate deficiency can also be a consequence of medical conditions that increase the need for folate or result in increased excretion of folate, including pregnancy, lactation, alcoholism, malabsorption, kidney dialysis, liver disease, certain anaemias and medications that interfere with folate metabolism.[2–4,8]

Folate deficiency is associated with several health risks. Overt folate deficiency leads to megaloblastic anaemia.[4,9] Suboptimal preconception folate intake increases risk of clinical spontaneous abortion, preterm birth,[9] low birth weight and neural tube defects.[2,10] Digestive disorders such as diarrhoea, loss of appetite and weight loss can occur with folate deficiency, as can weakness, sore tongue, headaches, heart palpitations, irritability, forgetfulness and behavioural disorders.[11] Additionally, evidence is emerging that folate deficiency may be implicated in the development of osteoporosis as a result of elevated homosysteine.[4] Thus, folate deficiency is an important public health issue, particularly in women of childbearing age.

More information about the magnitude of folate deficiency, especially in large, representative groups of individuals within countries, is needed.[1] In Ethiopia, protein and nutrient deficiencies are common and malnutrition is a grave concern[12-15] but information on the prevalence of folate deficiency is limited to one study of pregnant women.[13] To date, studies of nutrient deficiencies have been conducted only in select regions of this country. Thus, the first large assessment of women's nutritional status in Ethiopia was undertaken. In this study, we investigate the country-wide extent of folate deficiency and its risk factors. Information on the prevalence and magnitude of folate deficiency is needed to determine whether folate deficiency is a public health problem in Ethiopia and to contribute to global data on folate deficiency.

Methods

Study design

A cross-sectional, community-based study with an analytic component was conducted from June to July 2005 in nine of the

11 regions of Ethiopia, representing over 90% of the country's population, as previously described.[14] The study proposal was approved by the Research and Ethical Clearance Committee of the Ethiopian Health and Nutrition Research Institute (EHNRI). After the study had been explained to participants in their local languages, a written informed consent was obtained by thumbprint or signature from each subject. Subjects found positive for intestinal and haemo-parasites were treated immediately without charge.

Subjects and settings

Ethiopia, with an estimated population of 79 million, is situated in the horn of East Africa and is divided into 11 regions. The study sample was drawn from women of childbearing age, 15–49 years, living in 270 selected clustered villages across nine administrative regions of Ethiopia; two regions were inaccessible at the time of the study for security reasons.

Stratified and cluster sampling was used to identify the study subjects. The stratification was made based on different food staple diets. The samples were selected in two stages. In the first, 270 clusters were selected from the list of enumeration areas developed based on the sampling frame of the 1994 census. Then in the second stage, one village or site was randomly selected in each of the selected clusters. All women of childbearing age who were apparently in good health were invited to participate in the study. A total of 27 000 women, 100 from each of the 27 clusters, were recruited for the clinical assessment. Sample size was estimated based on a prevalence rate of anaemia of 18.7%, with a worst acceptable rate of 4.5%, and a 99% confidence level with a design effect of 2.[15] Of those recruited, 22 861 (86.7%) agreed to participate in the clinical assessment. A subsample of 1 143 (5%) was selected via a systematic sampling system for biological assessment and questionnaire administration; no further inclusion or exclusion criteria were considered for the subsample, but not all of these women had a sufficient blood sample to be included in all analyses. There were 970 women who completed all components of the study and were used in this assessment of folate deficiency.

Data quality

Prior to data collection, the health workers (doctors, nurses and medical technologists) drawn from the respective regions attended training sessions conducted by the two lead authors. The tools used in the study were adapted to the socio-cultural setting of the country through expert opinion. Before the actual survey took place, the questionnaire to collect demographic, health and dietary data was prepared in English and was pretested in the Oromia region where the training was conducted. After some corrections to the original tools had been made, the final version of the questionnaire was translated into the three main local languages spoken in the country, namely Amharic, Oromiffa and Tigrigna. The research staff followed protocols to standardise data collection methods. Supervisors from EHNRI checked data for completion and accuracy each day of the study. The data collected through the questionnaires were entered by experienced data entry clerks employed for this purpose. Thorough data cleaning followed data entry. A series of rules and ranges were used during data entry to identify incorrect responses or entry.

Demographic and health assessment

The trained doctors examined the subjects for pallor and other abnormalities, in other words illnesses, fatigue, dizziness, skin lesions and previous obstetric history, including birth defects or mortalities, and recorded their findings in the questionnaire. Socio-demographic characteristics and related medical and dietary history were collected by the trained nurses of the respective regions via interview.

Biological assessments

Blood collection was designed to permit determination of spot haemoglobin concentration and subsequently assess serum ferritin and folic acid status. The performance of the portable haemocues used in the study and microcuvettes were tested against a standard electronic counter (M530-coulter electronics Ltd., Miami) before commencing the study. Significantly high correlations ($r = 0.99$, $p < 0.0001$, $n = 30$) were observed between measurements of the two machines and a regression equation Hb-coulter = 1.11 + 0.99 for Hb-haemocue defined.

All biological samples were collected and analysed in duplicate and averaged. Stool and blood samples were collected aseptically in the morning before breakfast and then analysed partly at the site. Stool samples were examined for ova and parasites microscopically, as described by Kruger et al.[16]

Simultaneous venipuncture (cubital vein) and finger prick (capillary) samples were drawn for Hb determinations because finger prick-based haemocue determination tends to overestimate anaemia. The finger prick and vein samples had a similar result with the finger prick being slightly higher, but the mean difference was -1.12 ± 0.23g/L and was not statistically significant ($p = 0.1$). Significantly high correlation ($r = 0.96$, $n = 20$) and a regression equation Hb-capillary = 0.19 + 0.18 Hb-vein were demonstrated. Thus, haemoglobin was analysed using a portable haemoglobin meter HemoCue (HemoCue AB, Ängelholm, Sweden). After an adjustment for pregnancy and altitude,[17] anaemia was defined as haemoglobin < 11 g/dL in pregnant women and < 12 g/dL for non-pregnant women.

About 5 ml blood collected by venipuncture was carefully transferred into glass tubes without anticoagulants sealed with a rubber cap, stored in a cool box between four and eight degrees Celsius and transported to the respective laboratories of the regions for centrifugation and serum separation. Serum was stored frozen at -20 ^{0}C for about a week in each study site and then transported to EHNRI where it was analysed for serum folate and ferritin within six months after collection.

Both serum folate and ferritin concentration measurements were based on fully automated Elecsys 1020 analyser enzyme-linked immunosorbent assays (ELISA) technique. All other manufacturers' sample processing protocols for both nutrients were observed. Intra-laboratory check was based on batch samples analysed by two senior experts. A high correlation was obtained between the readings of both experts ($r = 0.99$; $n = 20$).

Serum folate was analysed using commercial kits purchased from Boerrhinger Mannheim, Germany, with quality control material purchased from Roche Company. Controls for the various concentration ranges were run as a single determination at

least once every 24 hours, once per repeated kit and after every calibration. There is no international standard for deficiency,[1] but the World Health Organization (WHO) recommends that 4 ng/mL be used as a cutoff for folate deficiency for serum folate based on metabolic indicators.[18] For this study, we used the cutoff values of serum folate ≤ 4 ng/mL and > 4 to 6.6 ng/mL for severe and marginal folate deficiency, respectively, while values of > 6.6 ng/mL were considered as optimal.[1,19]

Serum ferritin was analysed using an enzyme-linked immunosorbent assay with a fully automated Elecsys 1020, using commercial kits purchased from Boerrhinger Mannheim, Germany, at EHNRI. Iron deficiency was considered when serum ferritin was < 35 ug/L to balance the effect of infection, as recommended by the WHO for developing countries.[17] Iron deficiency anaemia was defined as haemoglobin ≤ 11 g/dL in pregnant women and ≤ 12 g/dL in non-pregnant women and serum ferritin ≤ 35 ug/L.

Dietary intake

Food patterns vary across Ethiopia. Staple crops can account for 90% of food intake[13] but vary by region. Staples are teff and cereals in the northern and central parts of the country; enset, cassava, maize (Zea mays), cereals and root crops in the south and southwest; and sorghum and maize in the east. For assessment of diet, vegetable, grain and animal product consumption in addition to the staple food intake was estimated using a simplified food frequency questionnaire (FFQ) previously used in Ethiopia. The FFQ data were collected by trained nurses via oral interview. The 20 foods in the FFQ included 14 plant-based foods (bananas, beans, bread, broccoli, cabbage, cassava leaves, ground nuts, morinaga, oranges, peanuts, potatoes, rice, spinach and Swiss chard), six animal products (beef, eggs, fish, liver, milk and poultry) and an "other"option.[14] Responses were given as number of times per day, week or month and then grouped according to frequency of consumption at once per week or more for animal products and once per day or more for plant foods. The differences in frequency for the plant and animal products were for both cultural sensitivity and practical reasons rather than as thresholds of nutritional adequacy. Animal products are consumed infrequently in Ethiopia due to both religious and economic reasons, and it would be offensive to ask how often animal products were consumed daily.

Statistical analysis

Statistical Package for Social Science version 12 was used for data analysis and cleaning. Standard tabulations were generated in which the outliers were identified prior to analysis. Pearson's chi-square test of independence was performed to determine the differentials of folate deficiency by explanatory variables, in other words haemoglobin, serum ferritin, anaemia, intestinal parasites, socio-demographic characteristics and number of times the mother consumed plant or animal products. Next, a stepwise backward logistic regression model was applied to test further the observed significant variables in bi-variate models while controlling for co-linearity. Odds ratio with 95% CI was also computed to assess the presence and degree of association among variables. In all analyses, P < 0.05 was considered significant.

Results

Of the 970 women participating in this study, 41.2% had formal education and 82.2% were married. The average age of the study sample was 32.6 ± 12.5 years, family size was 5.4 ± 2.1 persons and parity was 3.5 ± 1.0. Approximately half of the women had poor sanitary facilities. Demographic characteristics by region are presented in Table I.

Mean ± SD plasma folate was 5.57 ± 3.84 ng/mL (see Figure 1). Nearly half of the sample (46.1%) had severe folate deficiency (≤ 4 ng/mL) and 21.2% of the sample had marginal folate deficiency (> 4–6.6 ng/mL) while only 32.7% had optimal levels of serum folate

Figure 1: Folate status of women of reproductive age from nine regions of Ethiopia, 2005

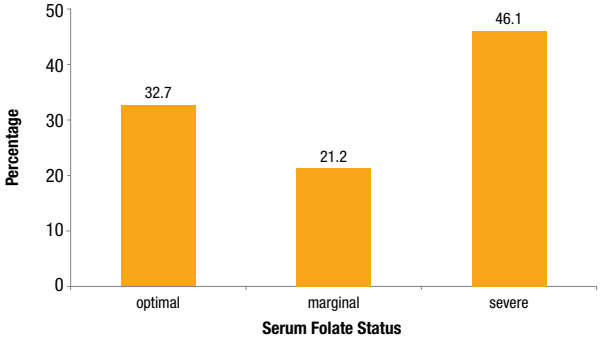

Table I: Demographic characteristics of Ethiopian women by region

Region	Number	Age, years Mean ± SD	Formal education[a] n (%)	Married n (%)	Family size Mean ± SD	Parity Mean ± SD	Lavatory facilities[b]
Afar	58	35.6 ± 7.5	6 (10.3)	52 (89.7)	5.8 ± 2.1	3.4 ± 1.1	55 (94.8)
Tigray	125	31.1 ± 8.9	49 (39.2)	58 (46.4)	5.8 ± 2.5	3.4 ± 1.1	81 (64.8)
Amhara	88	41.6 ± 8.6	9 (10.2)	80 (90.9)	4.8 ± 1.7	3.3 ± 0.8	38 (43.2)
Addis Ababa	126	29.2 ± 6.9	93 (73.8)	98 (77.8)	4.8 ± 1.8	2.9 ± 0.9	83 (65.9)
Oromiya	99	36.3 ± 10.9	54 (54.5)	88 (88.9)	5.8 ± 2.0	3.9 ± 1.0	28 (28.3)
SNNP[c]	119	34.4 ± 8.1	44 (37.0)	119 (100)	6.5 ± 2.2	4.0 ± 1.1	37 (31.1)
Benishangul-Gumuz	118	30.5 ± 7.1	29 (22.6)	108 (91.5)	5.9 ± 1.9	4.1 ± 1.1	59 (50.0)
Harari	135	36.5 ± 8.7	67 (49.6)	118 (87.4)	5.7 ± 2.1	3.7 ± 1.1	69 (51.1)
Dire-Dawa	102	28.5 ± 10.2	49 (48.0)	76 (74.5)	4.8 ± 1.9	3.5 ± 1.2	38 (37.3)
Overall	970	32.6 ± 12.5	400 (41.2)	797 (82.2)	5.4 ± 2.1	3.5 ± 1.0	488 (50.3)

[a] Primary education and above [b] Lavatory facilities available [c] South Nation and Nationalities People

Table II: Folate status in women of reproductive age by region from nine regions of Ethiopia, 2005

Region	Number	Folate status			X^2-value	p-value
		Severe deficiency[a] n (%)	Marginal deficiency[b] n (%)	Optimal folate status[c] n (%)		
Afar	58	34 (58.6)	11 (19.0)	13 (22.4)	4.4	0.1
Tigray	125	68 (54.4)	19 (15.2)	38 (30.4)	4.8	0.08
Amhara	88	29 (33.0)	22 (25.0)	37 (42.0)	6.6	0.03
Addis Ababa	126	28 (22.2)	25 (19.8)	73 (57.9)	46.2	0.001
Oromiya	99	50 (50.5)	5 (5.1)	44 (44.4)	18.7	0.001
SNNP[d]	119	40 (33.6)	48 (40.3)	31 (26.1)	29.7	0.00
Benishangul-Gumuz	118	35 (29.7)	44 (37.3)	39 (33.1)	24.1	0.001
Harari	135	109 (80.7)	17(12.6)	9 (6.7)	78.8	0.001
Dire-Dawa	102	54 (52.9)	15 (14.7)	33 (32.4)	3.4	0.1
Overall	970	447 (46.1)	206 (21.2)	317 (32.7)	20.3	0.001

[a] = ≤ 4 ng/mL [b] = > 4-6.6 ng/mL [c] = > 6.6 ng/mL [d] South Nation and Nationalities People

Table III: Distribution of characteristics associated with folate deficiency in women of reproductive age from nine regions of Ethiopia, 2005

Characteristic	Level	Number (% of sample)	Folate status			X^2-value	P-value
			Optimal	Severe deficiency[a]	Marginal deficiency[b]		
Formal education	No Yes	570 (58.8) 400 (41.2)	178 (31.2) 139 (34.7)	266 (46.7) 181 (45.3)	126 (22.1) 80 (20.0)	1.4	0.4
Age (years)	15–24 25–49	249 (25.6) 713 (74.4)	81 (32.5) 233 (32.7)	116 (46.6) 329 (46.1)	52 (20.9) 152 (21.3)	0.03	0.9
Marital status	Married Not married	797 (82.2) 173 (17.8)	316 (39.6) 46 (26.6)	349 (43.8) 98 (56.6)	117 (14.7) 29 (16.8)	11.8	0.002
Family size	Smaller (1–5) Extended (> 5)	403 (41.5) 567 (58.5)	143 (35.5) 173 (30.5)	176 (43.7) 272 (48.0)	84 (20.8) 122 (21.5)	2.7	0.2
Pregnant	Yes No	94 (9.7) 876 (90.3)	36 (38.3) 411 (46.9)	22 (23.4) 184 (21.0)	36 (38.3) 281 (32.1)	2.6	0.27
Parity	0–3 4–6	534 (55.1) 436 (44.9)	169 (31.6) 148 (33.9)	247 (46.2) 200 (45.9)	118 (22.1) 88 (20.2)	39.2	0.001
Birth spacing interval	≤ 2 years > 2 years	718 (74.0) 252 (26.0)	234 (32.6) 83 (32.9)	341 (47.5) 106 (42.1)	143 (19.9) 63 (25.0)	3.4	0.1
Oral contraceptive use	No Yes	449 (46.3) 521 (53.7)	159 (35.4) 158 (30.3)	184 (41.0) 263 (50.5)	106 (23.6) 100 (19.2)	8.8	0.01
Lavatory facilities	Open field latrine	488 (50.3) 482 (49.7)	148 (30.3) 169 (35.1)	237 (48.6) 210 (43.6)	103 (21.1) 103 (21.4)	2.9	0.2
Geographical region folate deficiency prevalence [c]	Low < 31% High > 31%	122 (12.6) 848 (87.4)	35 (28.7) 282 (33.3)	42 (34.4) 405 (47.8)	45 (36.9) 161 (19.0)	2.8	0.001
Illness in the previous two weeks [d]	Yes None	126 (13.0) 844 (87.0)	77 (61.1) 409 (48.5)	35 (27.8) 290 (34.4)	14 (11.1) 145 (17.2)	35.6	0.001
Intestinal parasites	No Yes	133 (13.7) 837 (86.3)	41 (30.8) 276 (33.0)	49 (36.8) 398 (47.6)	43 (32.3) 163 (19.52)	20.6	0.001
Meat, milk, egg consumption, weekly	≥ Once < Once	194 (20.0) 776 (80.0)	94 (48.5) 520 (67.0)	25 (12.9) 131 (16.9)	75 (38.7) 125 (16.1)	48.2	0.001
Vegetable, grain consumption, daily	≥ Once < Once	567 (58.5) 403 (41.5)	430 (75.8) 120 (25.3)	67 (11.8) 139 (34.5)	70 (12.3) 144 (35.7)	126.1	0.001
Haemoglobin status	Anaemic[e] Non-anaemic	295 (30.4) 675 (69.6)	169 (38.6) 203 (30.1)	58 (19.7) 302 (44.7)	68 (23.1) 170 (25.2)	74.8	0.001
Ferritin status	Iron deficient[f] Non-deficient	486 (50.1) 484 (49.9)	221 (45.5) 96 (19.8)	211 (43.4) 236 (48.8)	54 (11.1) 152 (31.4)	97.3	0.001

[a] Serum folate ≤ 4 ng/mL [b] Serum folate > 4–6.6 ng/mL [c] Prevalence of folate deficiency above or below 31% [d] Malaria, diarrhoea and pneumonia
[e] Haemoglobin < 11 g/dL in pregnant women and < 12 g/dL in non-pregnant women [f] Serum ferritin < 35 ug/L

Table IV: Factors contributing to folate deficiency among women assessed in nine regions of Ethiopia, 2005

Characteristic	Level	N	Overall FD[a]	COR (95%CI)	Adjusted OR (95% CI)
Marital status	Married	797	466	1	1
	Not married	173	127	1.4 (0.98–2.05)	1.3 (0.89–1.96)
Geographical region folate deficiency prevalence	Low, < 31%	848	443	1	1
	High, > 31%	122	80	1.2 (0.81–1.88)	1.2 (0.85–1.97)
Parity	0–3	534	365	1	1
	4–6	436	288	1.6 (0.83–3.12)	1.3 (0.97–1.89)
Oral contraceptive use	Yes	521	363	1.2 (0.96–1.64)	1
	No	449	290	1	0.8 (0.69–1.21)
Illness in the previous two weeks	Yes	126	49	1	1
	None	844	435	0.8 (0.63–1.18)	0.8 (0.60–1.12)
Intestinal parasites	No	133	92	1	1
	Yes	837	561	0.9 (0.61–1.34)	0.8 (0.55–1.27)
Meat, egg, milk consumption, weekly	≥ Once	194	100	1	1
	< Once	776	256	0.6 (0.38–0.97)*	0.8 (0.60–1.21)
Vegetable, grain consumption, daily	≥ Once	567	137	1	1
	< Once	403	283	0.7 (0.57–0.99)*	0.9 (0.72–1.2)*
Anaemia[b]	Yes	295	126	1	1
	No	675	472	1.04 (0.77–1.42)	0.8 (0.65–1.23)
Iron deficiency[c]	Yes	486	265	1	1
	No	484	388	1.6 (1.11–2.34)**	0.2 (0.17–0.34)**

[a] Serum folate ≤ 6.6 ng/mL [b] Haemoglobin < 11 g/dL in pregnant women and < 12 g/dL for non-pregnant women [c] Serum ferritin < 35 ug/L *p = 0.01; **p = 0.001

(> 6.6 ng/mL) (see Table II). All regions were significantly affected with folate deficiency except the Dire-Dawa, Tigray and Afar regions. Surprisingly, no elevated serum folate was observed (data not shown).

Thirteen per cent of the subjects had illnesses (pneumonia, diarrhoea and malaria), and 86.3% had intestinal parasites. In addition to the staple crop, 80% consumed meat, milk and eggs less than once a week and 41.5% ate vegetable and grains less than once a day. Thirty per cent of the women had anaemia and 50% were iron deficient. The occurrence of severe folate deficiency was significantly higher among unmarried women (p = 0.002), women with higher parity (p = 0.001), those who used oral contraceptives (p = 0.01), those who lived in regions where folate deficiency was prevalent (p = 0.001), those who were not ill (p = 0.001), those who had intestinal parasites (0.001), those who had meat, milk or eggs less than once a week (0.001), those who had vegetables and grains in addition to their staple crop less than once a day (0.001) those who were not anaemic (0.001) and those who did not have iron deficiency (0.001) (see Table III).

Next, a stepwise backward logistic regression model was applied to test further the observed significant variables (see Table III) in bi-variate models while controlling for co-linearity. In the logistic regression analysis, only low dietary intake of plant foods (p = 0.001) and iron deficiency (p < 0.001) retained their significance with regard to folate deficiency (see Table IV). The odds for developing folate deficiency was 0.9 times less likely among those with vegetable and grain consumption of more than once a day (AOR-0.9; 95%CI – 0.72–1.2) and 0.2 times less likely among those with adequate iron (AOR-0.2; 95%CI – 0.17–0.34). The odds of being folate deficient was 1.6 times higher among those who suffered from iron deficiency than their counterparts in the crude analysis; however, when the confounding effects of other variables were controlled, the observed association was reversed or negatively associated, implying substantial confounding with iron deficiency.

Discussion

The present study is the first of its kind to report the magnitude of folate deficiency in a broad sample of women of reproductive age across nine of the 11 regions of Ethiopia. The plasma folate concentration in our study was lower than that observed in women of childbearing age in countries with a high level of congenital anomalies[19] and many other international comparisons, including Nigeria and Zimbabwe,[1] but higher than levels reported in the United States of America.[1] A study done in neighbouring Sudan with pregnant women also showed higher levels of plasma folate.[20]

Likewise, the prevalence of folate deficiency was higher than in most of the countries that have national data.[1] On the other hand, in the only other study of folate status in Ethiopia, only 2% of a convenience sample of women who were in their third trimester of pregnancy and lived in rural southern Ethiopia had low plasma folate.[13] Nonetheless, our findings confirm that the Addis, Amhara and southern Ethiopia regions have among the lowest prevalence of folate deficiency within the country, yet even in these regions the prevalence of folate deficiency is substantially greater than 5%, which is indicative of a country-wide public health problem.[1] Thus, folate deficiency is clearly a concern throughout Ethiopia.

Our study suggests that folate deficiency is related to diet in Ethiopia. This finding is consistent with scientific consensus indicating that diet is one of the primary causes of folate deficiency.[1,2,4,5] The differences in food patterns in the country might help explain the disparities found in the different regions of the country. In Addis, the capital of the country and a more affluent area, food diversification is likely. Maize and fermented enset products are the major staple foods in the southern part of Ethiopia and animal products are consumed rarely. While it is expected that areas that rely on maize might have more folate deficiency, fermented foods may contribute some additional folate to the diet.[13, 20] The main dietary sources of folate are plant foods[3,18]; therefore, it is unsurprising that those women who eat plant foods less than once daily have higher levels of folate deficiency. In our sample, rates of folate deficiency varied with intake of animal

products. Meats, eggs and milk are relatively poor sources of folate and increased frequency of these foods would not protect against moderate folate deficiency. Liver is a better source of folate than most meats, but in Ethiopia liver is usually consumed together with other meat, thus its folate content is diluted. As decreased frequency of consumption of animal products was more commonly associated with severe folate deficiency, it could be that those women had diets limited in several micronutrients, including vitamin B_{12}. The diet consumed by most of the women in Ethiopia is generally poor and is likely deficient in several nutrients that interface with folate metabolism.[12,13] Also, it is unclear how food aversions and cravings noted during pregnancy in Ethiopia[21] may have impacted the dietary intake of those women who were pregnant, but there may have been a protective effect since the association between pregnancy and folate deficiency was not significant in our sample. The nature of our dietary assessment method limits a more exact measure of nutrient status, which would be beneficial. FFQs that estimate energy and nutrient intake more precisely are just being validated for Ethiopia[22] but, unfortunately, were not available at the time of this study.

Folate deficiency was related to iron deficiency but not anaemia in our study. The associations between folate deficiency and iron deficiency are not surprising, given the dietary intake of this population. The relationship with anaemia is less straightforward. It is well known that both folate and iron deficiencies can cause anaemia[3,13,18,23] and that concurrent iron and folate deficiency are frequently noted with anaemia,[7,17,24] particularly in pregnancy when an increased folate demand can lead to folate deficiency.[8–10] Despite this, Gibson et al[13] found that folate status did not predict haemoglobin in a group of pregnant women in southern Ethiopia. Likewise, Abdelrahim et al[20] found no relationship between folate deficiency and anaemia in their sample of pregnant Sudanese women although their sample had a relatively low prevalence of iron deficiency anaemia, unlike the women in our study. Anaemia is best tested by elevated mean corpuscular volume (MCV) in a complete blood count (CBC); however, this strategy is most effective in populations that have low prevalence of iron deficiency since iron deficiency tends to lower MCV more readily than it is raised by folate deficiency.[18] In addition, C-reactive protein (CRP) determination is helpful for interpretation of ferritin results since ferritin levels rise in cases of infection.[20] A limitation to our study is that due to logistical constraints (i.e. lack of a coulter counter at each site), it was not possible to do a CBC or CRP assessment that would have further clarified serum ferritin and anaemia results. It should be noted, however, that plasma folate did not differ relative to CRP status in a previous study conducted in Ethiopia.[13]

A further limitation to our study is that it was not possible to do analyses of vitamin B_{12} and/or homocysteine because of personnel shortages in all the study sites. These assessments would enhance our ability to distinguish folate deficiency from vitamin B_{12} deficiency. Additionally, we were not able to assess erythrocyte folate concentration, thus the present results might be underestimated.[1,3]

Also, while it is not likely because of no apparent systematic reasons, it is unknown whether any bias was introduced because some of the women who were selected to be in the study were not included because of inadequate amounts of blood for all analysis.

Besides relationships to diet and iron deficiency, marital status, parity, oral contraceptive use, illness and parasites were characteristics that were significant in folate deficiency. Since the consequences of folate deficiency can include atherosclerotic disease, cancer, cognitive impairment and depression,[18,19] it is important to investigate the risk factors for folate deficiency in Ethiopia further.

Conclusion

Folate deficiency is widespread in Ethiopia and is related to diet. The high prevalence of folate deficiency emphasises the need for sustainable folate intake through dietary diversification and appropriate public health interventions, such as supplementation during the periconceptional period and efforts to promote greater utilisation of maternal health care services.[25] Given that the plasma levels of folate are lower than are needed to support a healthy pregnancy, an investigation of the magnitude of neural tube defect and other pregnancy issues in the country is recommended.

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