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# Red blood cell folate level and associated factors of folate insufficiency among pregnant women attending antenatal care during their first trimester of pregnancy in Addis Ababa, Ethiopia

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## Abstract

**Objective:** Folate is an essential vitamin for de novo DNA synthesis and cell proliferation. Folate insufficiency at the time of conception and during the first trimester of pregnancy is associated with unintended pregnancy and birth outcomes, particularly neural tube defects. Hence, this study aimed to assess folate status and associated factors of folate insufficiency among pregnant women attending antenatal care during their first trimester of pregnancy in Addis Ababa, Ethiopia.
**Materials and methods:** A cross-sectional study was conducted from 8 August 2017 to 3 January 2018 in Addis Ababa. In this study, 160 participants were enrolled via the convenience sampling method. Red blood cell folate was measured by the electrochemiluminescence binding assay method. Data were entered into Epi-Data version 3.1 and analyzed by SPSS version 22.0. Descriptive statistics were used to describe demographic characteristics and to determine the magnitude of folate deficiency. Logistic regression was used to identify the risk factors for folate deficiency. A $p$-value of less than 0.05 was considered statistically significant.
**Results:** In this study, 44/160 (27%) participants had red blood cell folate level $<$400 ng/mL, insufficient to prevent neural tube defect. Multivariate regression showed that regular vegetable consumption was an independent determinant factor for red blood cell folate level (adjusted odds ratio: 0.41, confidence interval: 0.18–0.93).
**Conclusion:** This study shows that a large magnitude of the first-trimester pregnant women had red blood cell folate concentrations below levels that are maximally protective against neural tube defects. Folic acid supplementation and supplemental nutrition containing green leafy vegetables should be promoted during the periconceptional period. In addition, the policymakers should set rules for mandatory folic acid fortification.



## Keywords

Folate metabolism, folate insufficiency, pregnant women, determinant factors, neural tube defect

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## Introduction

Some vitamins, such as folate and B12, are vital for body cell growth and development. Folate is known as vitamin B9, which plays a metabolic role as a carrier for one carbon unit. The one-carbon groups on folate (FH4) may be oxidized or reduced and then transferred to other compounds. This process involves the synthesis of glycine from serine, base thymine required for DNA synthesis, and purine bases required for both DNA and RNA synthesis. It is also essential for the transfer of methyl groups to vitamin B12 to produce

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methionine from homocysteine. Methionine is important for DNA, protein, and lipid methylation.[1,2]

Inadequate intake of folate, either through the consumption of folate-rich foods or supplements, affects folate status. In addition, intestinal malabsorption, conditions causing increased cell proliferation, antifolate drugs, and common gene mutations (e.g. methylenetetrahydrofolate reductase C677T polymorphism) can contribute to folate deficiency. Chronic hemolytic anemia, pregnancy, lactation, and rapid growth during adolescence can result in an increased folate requirement.[3–5] Folate interaction with vitamins B6, B12, and zinc through the methylation process of the one-carbon metabolism also affects blood folate status.[6,7]

A deficiency of folate reduces thymidylic acid synthesis that produces megaloblastic anemia and birth defects, particularly neural tube defects (NTDs). Folate deficiencies also cause hyperhomocysteinemia, which can lead to atherosclerosis formation, and disturbances of methylation, leading to effects on the nervous system and other organs.[8]

Folate deficiency is the second cause of anemia during pregnancy, following iron deficiency, due to increased requirement and dietary restriction. However, the global magnitude of the problem is unavailable due to the under-representation of pregnant women in study surveys.[9,10] The burden of folate deficiency is fluctuating in different countries. The prevalence of folate deficiency in high-income countries is generally reported as less than 5%[10–12] with prevalence rates for women during their first trimester of pregnancy decreasing from about 55% to 1% after the fortification of flour with folic acid.[10,13] However, folate deficiency is a public health problem in many low- and middle-income countries[10] with rates of deficiency in most low-income countries being reported as higher than 20%.[11] However, folate deficiency is a public health problem in low-income and middle-income countries.[10] The prevalence of folate deficiency in most low-income countries is more than 20%.[11] In Côte d'Ivoire, the prevalence of folate deficiency (<4 ng/mL) among women of reproductive age was 86%.[12] In addition, in neighboring eastern Sudan, serum folate deficiency was reported in 57.7% of pregnant women.[13] A cross-sectional study in women of reproductive age in nine administrative regions of Ethiopia reported 46% to severe folate deficiency (⩽4 ng/mL) and 21.2% (>4–6.6 ng/mL) marginal folate deficiency. In addition, the Ethiopian public health institute national survey on micronutrient deficiencies showed red blood cell (RBC) folate deficiencies (RBC folate determined by microbiological assay method and a cutoff <140 ng/L) in 32% and 27% of non-pregnant reproductive age women in Ethiopia and Addis Ababa, respectively.[14,15]

Folate insufficiency at the time of conception and early pregnancy is associated with increased risks for NTDs in the developing fetus, intrauterine growth retardation, preeclampsia, and early miscarriage.[16,17] In addition, inappropriate treatment of B12 deficiency with folate can cause nerve degeneration.[18] Therefore, measuring the level of both vitamins and supplementing accordingly is critical for preventing NTD and other congenital abnormalities.[19]

Ethiopia has implemented a folic acid/iron supplementation program for pregnant women since 2008.[20] However, folate-fortified foods are unavailable in the food of Ethiopia.[21] The burden of NTD cases in Ethiopia is significantly higher than that in other sub-Saharan African countries.[22] The incidence of NTD cases in different regions of Ethiopia ranges from 131 to 25.7 per 10,000 births[23–26] Due to these reasons, the city's population is at a higher risk of folate deficiency that can lead to an increased occurrence of NTD. Unfortunately, no systematic study has been undertaken so far in Addis Ababa to understand whether folate deficiency is a public health problem in first-trimester pregnant women in causing NTD in the developing fetus. Therefore, this study assessed the prevalence and determinant factors of folate deficiency among first-trimester pregnant women.

## Materials and methods

### *Study area and period*

This study was conducted from 8 August 2017 to 4 January 2018 at T/Haimanot and Lideta health centers (HCs), located in Lideta sub-city, Addis Ababa, Ethiopia. T/Haimanot HC is located 500 m north of Tikur Anbessa Specialised Hospital and Lideta HC is located behind Saint Lideta Church. According to the antenatal care (ANC) registry books of the corresponding HCs, 270 and 500 first-trimester pregnant women visited ANC units of T/Haimanot and Lideta HCs per year, respectively. Both HCs provide ANC, prevention of mother-to-child transmission, delivery, and referral services for pregnant women in their maternal health department.

### *Study design*

An institutional-based cross-sectional study design was used.

### *Population*

All pregnant women who were visiting T/Haimanot and Lideta HCs were the source population for the study. The study population for this study was all first-trimester pregnant women who were attending ANC units of T/Haimanot and Lideta HCs during the study period and met the eligibility criteria.

### *Eligibility criteria*

*Inclusion criteria.* All first-trimester pregnant women who were aged 18 years and older and attending ANC in the HCs during the study period and voluntarily participated in the study were included.

*Exclusion criteria.* Conditions that can increase or decrease folate level abnormally, such as women on antidiabetic, anti-cancer, anti-tuberculosis, anticonvulsants, and anti-malaria drugs. Women diagnosed with cancer and women with any major bleeding and blood transfusion in the previous 3 months were excluded from this study.

## Sample size determination and sampling procedure

The single population proportion formula with a 95% confidence interval (CI) was used to determine the sample size of the study. Previously, a systematic review of data from 2005 to 2015 on the micronutrient status of reproductive age and pregnant women in Ethiopia, Kenya, Nigeria, and South Africa showed 3%–12% folate deficiency during pregnancy[27]

$$n = \frac{\left(z1 - \frac{\alpha}{2}\right)^2 p(1-p)}{d^2}$$

where $n$ is the minimum sample size required; $z1-\alpha/2$ is the standard normal variable at $(1-\alpha)\%$ confidence level and $\alpha$ (level of significance). Usually, 95% confidence level is used, which is 1.96; $p$ is the estimate of the prevalence rate of folate (12%) deficiency during pregnancy. In the population; $d$ is the margin of sampling error tolerated, assumed to be 0.05

$$n = \frac{(1.96)^2\ 0.12(0.88)}{0.05^2} \approx 162$$

Based on this, the sample size of the study was 162.

Five HCs are found in the Lideta sub-city. Out of these, we selected two HCs using the random sampling method. The number of study units for each HC was proportionally allocated based on the number of first-trimester pregnant women coming per year. Those who were part of the final sample size were selected purposively based on the caseload until we obtained the sample size. Out of these, two participants were excluded from the study due to an ineligible blood sample.

## Study variables

The independent variables for this study were Socio-demographic characteristics, obstetric and dietary habit. The dependent variable was RBC folate concentration.

## Data and blood sample collection procedures

Socio-demographic characteristics and other data related to pregnancy and lifestyle were collected from the participants via face-to-face interviews using an Amharic language version structured questionnaire by experienced midwifery professionals. Furthermore, about 5 mL of venous blood was collected from 162 study participants, with 160 usable blood samples for RBC folate analysis, by skilled laboratory technologists in both HCs. Blood was collected in an ethylenediaminetetraacetic acid (EDTA) tube. The collected sample was immediately processed. RBC hemolysate sample was prepared by mixing 100 µL of whole blood with freshly prepared 1 mL 1% ascorbic acid. Aliquots of RBC hemolysate were frozen-stored at −80°C until analysis. Safety precautions were taken while handling blood and disposing of it.

## Measurement of RBC folate concentrations

There are several methods to assay and calculate folate RBC concentration. Some of these methods are used in the medical field and others in the pharmacological studies.[28] In this study, RBC folate was measured by the electrochemiluminescence binding assay method using the Elecsys folate RBC assay analyzer that employs a competitive test principle using natural folate-binding protein (FBP) specific for folate. Folate in the sample competes with the added folate (labeled with biotin) for the binding sites on FBP.[29]

Whole blood treated with anticoagulant (EDTA) mixed with 1% ascorbic acid solution was incubated for liberation and stabilization of the intracellular folate. The hemolysate sample was first incubated with the folate pre-treatment reagents; bound folate was released from endogenous FBPs. Then, the pretreated sample was incubated with ruthenium-labeled FBP, a folate complex is formed. Afterward, streptavidin-coated microparticles and folate labeled with biotin were added to form a ruthenium-labeled FBP–folate biotin complex. Finally, the reaction mixture was aspirated into the measuring cell where the microparticles were magnetically captured onto the surface of the electrode. The application of a voltage to the electrode then induces a chemiluminescent emission, which was measured by a photomultiplier.[29]

Results were determined via a calibration curve which was specifically generated by the instrument by two-point calibration and a master curve provided via the reagent barcode. The analyzer automatically calculates the analyte concentration of each sample in ng/mL from the calibration curve. Then, folate concentration in the erythrocyte fraction of the sample (RBC folate) was calculated using the following equation

$$\text{RBC folate } = \frac{\text{analyzer result}}{\text{\% hematocrit}} \times 100$$

Note: Hematocrit was measured during the sample collection from fresh whole blood.

The cut-off value for the lower limit of normal folate level is still controversial across the pieces of literature.

In this study, the concentration suggested to achieve the greatest reduction of NTDs is based on the information provided by World Health Organization (WHO), by which RBC folate <905 nmol/L (<400 ng/mL) is defined as the RBC folate threshold assessed using microbiological assay method that can be used as an indicator of folate insufficiency in women of reproductive age at the population level.[11,30]

### Statistical analysis

The data were entered using Epi-Data statistical software version 3.1 and then exported to SPSS software version 22.0 for analysis. Simple descriptive statistics, such as mean, standard deviation, median, frequency, percentiles, and percentage, were used to present socio-demographic characteristics and magnitude of RBC folate level. Logistic regression was used to analyze independent variables with folate deficiency, and multivariate logistic regression was used to identify the independent associated factors of folate deficiency. A variable with a $p$-value of $\leqslant 0.5$ in bivariable logistic regression was eligible for multivariable logistic regression analysis model. A $p$-value of less than 0.05 was considered statistically significant in all analyses.

### Data quality control and management

The socio-demographic characteristics and related data were collected by nurse professionals. The questioner was adapted from the Ethiopian public health institute (EPHI) national micronutrient survey report.[15] The questionnaire was pre-tested in randomly selected 5% populations in other health facility (Abinet health center) to identify potential challenges related to the survey questioner.[31] Blood sample collection, processing, and laboratory analysis were handled by trained laboratory technologists. Elecsys Folate RBC assay analyzer was used to measure and analyze the test. Data coding, entering, verifying, and cleaning were performed with great care.

### Ethical consideration

This study was conducted as per the Declaration of Helsinki. Before starting data collection and preliminary study, an ethical clearance letter with reference number SOM/BCHM/146/2009 was obtained from the Departmental Research and Ethics Review Committee, Department of Biochemistry, College of Health Science, Addis Ababa University. Official collaboration letters were obtained from each HC. The purpose of the study was briefly explained to the study participants and they were informed that their responses would be treated with strict confidentiality. Samples and data were collected after the study participants gave full written consent. Confidentiality, anonymity, neutrality, accountability, and academic honesty were maintained throughout the study.

### Operational (working) definitions

- Folate conversion factor: nmol/L × 0.44 = ng/mL, ng/mL × 2.27 = nmol/L.
- First-trimester pregnancy: Time extending from the first day of the last menstrual period through 12 weeks of gestation.
- Folate insufficiency for NTD prevention at the population level: RBC folate level <400 ng/mL or <906 nmol/mL, which was established based on RBC folate assessed using microbiological assay method with folic acid as the calibrator.
- Gravida: The total number of confirmed pregnancies that a woman has had, regardless of the outcome.
- Parity: The number of times a woman has given birth, alive or dead.
- Regular dietary intake: continues the consumption of diet type on a daily, weekly, or monthly basis.

## Results

### General characteristics

A total of 162 first-trimester pregnant women attending ANC in Lideta and T/Haimanot HCs participated in this study. From which the blood sample of two participants was not usable and was excluded. Among all participants, 79 (49.4%) were in the age group of 25–29 years, 149 (93.1%) were married, 125 (79.1%) were formally educated, and 82 (51.2%) were employed. In addition, 72/160 (45%) participants were pregnant for the first time. From 88 participants, who had given birth before, 49 (59%) of them waited 3 or fewer years between pregnancies. Moreover, 2 (2.27%) participants had a history of NTD (Table 1).

### Dietary intake history

A total of 160 participants were asked about their consumption habits on some types of foods; the types of foods asked and the magnitude of participants who reported that they consumed them regularly on a daily, weekly, and monthly basis were fruit: 104 (65%), green leafy vegetable: 110 (68.8%), grain: 160 (100%), milk: 94 (58.8%), meat: 89 (55.6%), and egg: 62 (38.7%) (Table 2).

### Prevalence of folate insufficiency

Among 160 participants for whom RBC folate concentration was measured, 44/27.5% (95% CI: 21.4%–35.9%) had folate RBC folate level <400 ng/mL (insufficiency for NTD prevention).

### Maternal characteristics associated with folate deficiency

Bivariate logistic regression shows that income was significantly associated with RBC folate, and participants with a

**Table 1.** General characteristics of first-trimester pregnant women attending ANC at selected HCs in Addis Ababa, Ethiopia, 2017/18.

| Variables | | No. | % |
|---|---|---|---|
| Age (years) | 18–24 | 45 | 28.1 |
| | 25–29 | 79 | 49.4 |
| | ⩾30 | 36 | 22.5 |
| Marital status | Married | 149 | 93.1 |
| | Single | 11 | 6.9 |
| Formal education | No | 35 | 21.9 |
| | Yes | 125 | 79.1 |
| Occupation | Employed | 82 | 51.2 |
| | Not employed | 78 | 48.8 |
| Monthly income | Low | 54 | 35.1 |
| | Middle | 55 | 35.7 |
| | High | 45 | 29.2 |
| No. of pregnancy | Once | 72 | 45.0 |
| | More than once | 88 | 55.0 |
| Parity | Once or no | 138 | 86.3 |
| | Multipara[a] | 22 | 17.8 |
| Pregnancy interval (years) | ⩽3 | 126 | 78.8 |
| | >3 | 34 | 11.3 |
| No. of children | ⩽1 | 136 | 85.0 |
| | ⩾2 | 24 | 15.0 |
| History of previous NTDs[a] | Yes | 2 | 2.3 |
| | No | 86 | 97.7 |

ANC: antenatal care; ETB: The Ethiopian birr; Parity: The number of times a woman has given birth, alive or dead; No of pregnancy: The number times the woman has been pregnant.

[a]History of previous NTD is taken from 88 participants, who had given birth before

**Table 2.** Regular dietary intake history of first-trimester pregnant women in first-trimester pregnancy, Addis Ababa, Ethiopia, 2017/2018.

| | | Variables | | | | | |
|---|---|---|---|---|---|---|---|
| | | Fruit | Green leafy vegetable | Grain | Milk | Meat | Egg |
| Dietary intake | | *n* (%) | *n* (%) | *n* (%) | *n* (%) | *n* (%) | *n* (%) |
| Yes | Daily | 7 (6.7) | 12 (10.9) | 94 (58.7) | 22 (23.4) | 4 (5.6) | – |
| | Weekly | 92 (88.5) | 93 (84.5) | 66 (41.3) | 71 (75.5) | 58 (81.7) | 57 (91.9) |
| | Monthly | 5 (4.8) | 5 (4.5) | – | 1 (1.1) | 9 (12.7) | 5 (8.1) |
| | Total | 104 (65) | 110 (68.8) | 160 (100) | 94 (58.8) | 89 (55.6) | 62 (38.7) |
| No | | 56 (35) | 50 (31.2) | – | 66 (41.2) | 71 (44.4) | 98 (61.3) |

Regular dietary intake (yes): continues consumption of diet type on a daily, weekly, or monthly basis.

low income had 4.03 times higher odds of having an RBC folate level <400 ng/L compared to participants with high income. By the multivariate analysis, when the number of pregnancies, income, fruit, vegetable, and milk intake were included in the model, vegetable intake (adjusted odds ratio (OR): 0.41, CI: 0.18–0.93) was the only determinant factor associated with folate insufficiency (Table 3).

## Discussion

This study assessed the magnitude of folate insufficiency and associated factors among pregnant women in their first trimester. In this study, the magnitude of folate insufficiency was 27.5% among pregnant women in their first-trimester pregnancy. This finding was comparable with other studies conducted in Addis Ababa, Ethiopia, 25.3% (RBC folate determined by microbiological assay method and a cut-off <140 ng/L)[15] and 22% (serum folate ⩽4 ng/mL).[14] However, this finding was lower than a study reported in a national prevalence among reproductive age group in Ethiopia which is 46% (serum folate ⩽4 ng/mL).[16] The lower folate deficiency (the higher folate level) of women living in Addis Ababa compared to the national prevalence might be due to the easy access to food and health facilities. Evidence

**Table 3.** Factors associated with folate insufficiency during the first trimester of pregnancy, Addis Ababa, Ethiopia, 2017/2018.

| Variables | | RBC folate⩾400 ng/mL N (%) | RBC folate <400 ng/mL N (%) | Crude OR OR (95% CI) | Adjusted OR OR (95% CI) |
|---|---|---|---|---|---|
| Age (years) | 18–24 | 34 (29.3) | 11 (25.0) | 0.84 (0.31–2.28) | – |
| | 25–29 | 56 (48.3) | 23 (52.3) | 1.07 (0.45–2.56) | – |
| | ⩾30* | 26 (22.4) | 10 (22.7) | 1 | – |
| Formal education | No | 25 (21.6) | 10 (22.7) | 1.07 (0.47–2.46) | – |
| | Yes* | 91 (78.4) | 34 (77.3) | 1 | – |
| Occupation | Employed | 59 (50.9) | 23 (52.3) | 1.06 (0.53–1.12) | – |
| | Not employed* | 87 (49.1) | 21 (47.7) | 1 | – |
| Marital | Married | 108 (93.1) | 40 (90.9) | 0.74 (0.21–2.6) | – |
| | Single* | 8 (6.9) | 4 (9.1) | 1 | – |
| Income | Low | 31 (27.9) | 23 (53.5) | 4.03 (1.53–10.6)¶ | 2.39 (0.79–7.28) |
| | Middle | 42 (37.8) | 13 (30.2) | 1.68 (0.61–4.65) | 1.41 (0.49–4.09) |
| | High | 38 (34.2) | 7 (16.3) | 1 | – |
| No. of pregnancy | Once | 53 (45.7) | 19 (43.2) | 0.90 (0.45–1.82) | – |
| | More than once | 63 (54.3) | 25 (56.8) | 1 | – |
| Parity | Once or no | 101 (87.1) | 37 (84.1) | 1.27 (0.48–3.4) | – |
| | Multipara* | 15 (12.9) | 7 (15.9) | 1 | – |
| No. of children | ⩽1 | 100 (86.2) | 36 (81.8) | 0.72 (0.28–1.82) | – |
| | ⩾2* | 16 (13.8) | 8 (18.2) | 1 | – |
| Pregnancy interval (yrs) | ⩽3 | 31 (54.4) | 17 (77.3) | 2.85 (0.93–8.79) | – |
| | >3 | 28 (45.5) | 5 (22.7) | 1 | – |
| Nutritional intake | | | | | |
| Fruit | Yes | 83 (71.6) | 23 (52.3) | 0.44 (0.21–0.89)¶ | 0.60 (0.28–1.33) |
| | No* | 33 (28.4) | 21 (47.7) | 1 | 1 |
| Meat | Yes | 52 (44.8) | 19 (43.2) | 1.43 (0.71–2.29) | |
| | No* | 64 (55.2) | 25 (56.8) | 1 | – |
| Vegetable | Yes | 86 (74.1) | 23 (52.3) | 0.38 (0.19–0.79)¶ | 0.41 (0.18–0.93)¶ |
| | No* | 30 (25.9) | 21 (47.7) | 1 | 1 |
| Milk | Yes | 74 (63.8) | 20 (45.5) | 0.47 (0.23–0.98)¶ | 0.49 (0.22–1.07) |
| | No* | 42 (36.2) | 24 (54.5) | 1 | 1 |

RBC: red blood cells; OR: odds ratio; CI: confidence Interval; ETB: The Ethiopian Birr, yrs: years.
*Reference, statistically significant ($p < 0.05$).

showed that women who got an additional source of folate presented a lower prevalence of folate deficiency.[32]

However, this study found a lower percentage of folate insufficiency compared to studies conducted in Belgium 39% (RBC folate less than 400 ng/mL),[33] Côte d'Ivoire (serum folate <6.8 ng/mL) (86%),[13] and Eastern Sudan 57.7%.[12] This difference might be due to the habit of fermented food consumption in Ethiopia since more folates can be produced through fermentation.[34,35]

In contrast, the prevalence of folate deficiency in the first trimester of pregnancy in high-income countries has decreased from 55% to 1% after the era of folic acid fortification of flour.[10,36] In addition, in Turkey, only 0.5% of the first-trimester pregnant women were found to have folate deficiency (serum folate <3 ng/mL).[37] Those findings showed a lower prevalence compared in our study. Thus, the higher prevalence in our study might be due to the unavailability of effective folic acid-fortified foods and fortification policy.[21] Generally, this study defines that folate deficiency is still a continuous public health problem in the first-trimester pregnant women of Addis Ababa that can lead to an increased risk of NTD occurrence.

In this study, vegetable intake was an independent determinant factors associated with folate insufficiency. Similarly, another study reported that lower intake of green leafy vegetables was the main determinant factor of folate insufficiency.[38] In addition, another study in Ethiopia found an association between folate deficiency and a lower intake of a plant product–containing diet.[14] These findings can be explained by the fact that folates are found in green leafy vegetables.[39] Therefore, frequent intake of vegetables may lower the likelihood of folate deficiency among pregnant women.

## Limitations of the study

This study did not include other clinical biomarkers that are important to investigate the risk of NTD, such as vitamin B12, iron, and homocysteine levels, due to the unavailability of resources including reagents. The quantity of food consumption habit was not taken under consideration. Comparisons between studies were difficult because of the differences in the RBC folate assay methods and cut-off values. This study was also prone to selection bias due to the challenges to get an adequate number of women in their first trimester.

## Conclusion

This study shows that a large magnitude of the first-trimester pregnant women had RBC folate concentrations below levels that are maximally protective against NTDs. Folic acid supplementation and supplemental nutrition containing green leafy vegetables should be promoted during the periconceptional period. Also, the policymakers should set rules for mandatory folic acid fortification. In addition, a longitudinal prospective study that includes other biomarkers such as vitamin B12, iron, and homocysteine should be conducted to identify determinants of NTDs risks in Ethiopia.

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### Author contributions

All authors made a significant contribution to the work reported, whether in the conception, study design, execution, acquisition of data, analysis, and interpretation or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

### Ethics approval

Ethical approval for this study was obtained from the Departmental Research and Ethics Review Committee, Department of Biochemistry, College of Health Science, Addis Ababa University with reference number SOM/BCHM/146/2009.

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### Informed consent

Official collaboration letters were obtained from each health centers. Written informed consent was obtained from all subjects before the study.

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### Supplemental material

Supplemental material for this article is available online.

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