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Folate and Vitamin B12 Status in Women of Reproductive Age in Rural Haryana, India: Estimating Population-Based Prevalence for Neural Tube Defects

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Abstract

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Supporting Information
Additional supporting information can be found online in the Supporting Information section.

Conflicts of Interest
The authors declare no conflicts of interest.

Background: Folate and vitamin B12 deficiencies in pregnant women are associated with increased risk for adverse maternal and infant health outcomes, including neural tube defects (NTDs).

Methods: A population-based cross-sectional survey was conducted in two rural areas in Ambala District, Haryana, India in 2017 to assess baseline folate and vitamin B12 status among women of reproductive age (WRA) and predict the prevalence of NTDs. We calculated the prevalence of folate and vitamin B12 deficiency and insufficiency by demographic characteristics among 775 non-pregnant, non-lactating WRA (18–49 years). Using red blood cell (RBC) folate distributions and an established Bayesian model, we predicted NTD prevalence. All analyses were conducted using SAS-callable SUDAAN Version 11.0.4 to account for complex survey design.

Results: Among WRA, 10.1% (95% CI: 7.9, 12.7) and 9.3% (95% CI: 7.4, 11.6) had serum (<7 nmol/L) and RBC folate (<305 nmol/L) deficiency, respectively. The prevalence of RBC folate insufficiency (<748 nmol/L) was 78.3% (95% CI: 75.0, 81.3) and the predicted NTD prevalence was 21.0 (95% uncertainly interval: 16.9, 25.9) per 10,000 live births. Prevalences of vitamin B12 deficiency (<200 pg/mL) and marginal deficiency (≥200 pg/mL and ≤300 pg/mL) were 57.7% (95% CI: 53.9, 61.4) and 23.5% (95% CI: 20.4, 26.9), respectively.

Conclusions: The magnitude of folate insufficiency and vitamin B12 deficiency in this Northern Indian population is a substantial public health concern. The findings from the survey help establish the baseline against which results from future post-fortification surveys can be compared.

Keywords

India; neural tube defects; red blood cell folate; serum folate; serum vitamin B12; women of reproductive age

1 | Introduction

Folate and vitamin B12 deficiencies in pregnant women are associated with increased risk for adverse maternal and infant health outcomes, including neural tube defects (NTDs) (World Health Organization 2015a, 2015b; Finkelstein, Layden, and Stover 2015; Bailey and Hausman 2018; Ray et al. 2007; Molloy et al. 2008, 2009; Molloy 2018). Neural tube defects are severe birth defects of the brain and spine due to failure of neural tube closure by the 28th day of conception and can lead to fetal and infant deaths, as well as lifelong disabilities (Cordero et al. 2015; Centers for Disease Control and Prevention 1992; Crider et al. 2022).

It is estimated that over 260,000 infants with NTDs are born every year globally (prevalence: 18.6 per 10,000 live births), with higher prevalence in low and middle-income countries (Blencowe et al. 2018; Zaganjor et al. 2016; Lo, Polšek, and Sidhu 2014; Atta et al. 2016). The prevalence of NTDs in India, estimated to be 41–45 per 10,000 births, is among the highest globally, with wide variations across the country ranging from 5 per 10,000 births in Maharashtra to 182 per 10,000 births in Haryana (Bhide et al. 2013; Allagh et al. 2015; Sharma and Gulati 1992; Taksande et al. 2010).

Since NTDs occur in the early weeks of pregnancy, often before women know they are pregnant, it is important that women have adequate folate levels periconceptionally. Evidence has shown that the use of folic acid supplements during the periconceptional period can decrease the risk for NTDs (Smithells, Sheppard, and Schorah 1976; MRC Vitamin Study Research Group 1991; Czeizel and Dudás 1992; Berry et al. 1999). Multiple countries have been able to increase consumption of folate among women of reproductive age (WRA) through folic acid fortification of centrally produced staples (e.g., wheat flour); studies from these countries showed a substantial reduction (31%–78%) in the prevalence of NTDs post-fortification (Das et al. 2013; Cordero, Do, and Berry 2008; López-Camelo et al. 2005; Sayed et al. 2008; Calvo and Biglieri 2008; Williams et al. 2002; Liu et al. 2004; Persad et al. 2002; De Wals et al. 2003; Ray et al. 2002). Several meta-analyses of pre- and post-fortification prevalence of NTDs among different countries showed consistent results, a 41%–46% reduction in prevalence depending on the fortification dosage, intake, and baseline NTD prevalence (Blencowe et al. 2010; Keats et al. 2019; Crider et al. 2022). While micronutrient food fortification can be a successful public health approach to address micronutrient deficiencies, India currently does not have a national policy on fortification of centrally distributed staples with folic acid, vitamin B12, or iron. Micronutrient supplementation (e.g., iron and/or folic acid) has had limited success since the prevalence of micronutrient deficiencies, especially anemia (prevalence: 57% in women aged 15–49 years), remains high (Ministry of Health & Family Welfare, Government of India 2022). In Haryana, there is a high prevalence of anemia, folate deficiency, and vitamin B12 deficiency. The National Family Health Survey (NFHS-5) 2019–21 found that almost 61% of non-pregnant women aged 15–49 years in Haryana were anemic while another study found that 72% of adolescent girls in rural Haryana had anemia (Ministry of Health & Family Welfare, Government of India 2022; Subramanian et al. 2022). A study in one of the districts in Haryana found that 37% of the study participants (males and females aged 30–65 years) were folate deficient, and 46% of the participants were vitamin B12 deficient (Yadav, Thakur, and Kiranmala 2018).

Since the World Health Organization (WHO)’s establishment of the population-level red blood cell (RBC) folate threshold for the optimal prevention of NTDs among WRA in 2015, several countries were able to apply the WHO guidelines to population-based data to assess the prevalence of RBC folate insufficiency and predict NTD prevalence (World Health Organization 2015a; Cordero et al. 2015; Crider et al. 2014, 2018; Tinker et al. 2015; Rosenthal et al. 2016; Finkelstein et al. 2021). We use the same methodology to predict NTD prevalence in this study.

To address micronutrient deficiencies, multi-sector partners representing public, private, academic, and civic sectors collaborated beginning in 2017 on a large-scale wheat flour fortification demonstration project through social safety net programs in Haryana, India. The objectives of this study were to conduct a population-based household and biomarker survey in rural Ambala District, Haryana to assess the baseline folate and vitamin B12 status in WRA, as part of the fortification demonstration project and to predict NTD prevalence. Findings from this survey will serve as a baseline for future comparisons.

2 | Methods

2.1 | Ambala District and Population

Ambala District is one of 22 districts in Haryana, located in the northeastern part of the state. According to the 2011 Census, Ambala District had a population of 1,128,350 with 46.9% women, 84.7% Hindus, 2% Muslims, and 12.3% Sikhs. About 56% of the population lived in rural areas (Population Census 2011, 2011).

2.2 | Study Design and Sample Selection

A population-based cross-sectional survey was conducted among non-institutionalized, non-pregnant women of reproductive age (18–49 years) in two rural blocks (sub-districts)—Naraingarh and Barara in Ambala District in 2017. To select a representative probability sample of households, a multi-stage probability proportional to size (PPS) sampling was used (Henderson and Sundaresan 1982). In the first stage of sampling, 52 villages were selected using PPS based on the sampling plan of 55 clusters. In the second stage, enumeration blocks (EB) in each of the selected villages were selected randomly. One EB was chosen from each of the 52 selected villages, except for three villages from which two EBs were selected. A total of 55 EBs were selected in the second stage. In the third stage, 21 houses were selected randomly within each selected EB. If there was more than one household residing in the selected house, the interviewer would randomly select one household to participate. A household is defined as "a group of persons who normally live together and take their meals from the same kitchen (same cooking pot) unless the exigencies of work prevent any of them from doing so" (Population Census 2011, 2011). The interviewers then identified the head of household and all eligible women (non-pregnant women aged 18–49 years) residing in the household. When two or more eligible women were identified in a single household, one woman was chosen through a random selection process. If the selected household was not available at initial contact, two subsequent visits were made to check for availability before labeling the household as unavailable/nonresponsive. The same procedure was used for the selected woman, that is, if the selected woman was not available at initial contact, two more attempts were made on different occasions before labeling her as nonresponsive.

A total of 1148 households were needed to estimate a 50% prevalence of folate insufficiency or vitamin B12 deficiency and marginal deficiency at baseline and an expected post-intervention decrease in prevalence of 10%. The following parameters were used in the calculation of the minimum household sample size: study power: 80%, survey design effect: 2.0, household response rate: 90%, and response rate among non-pregnant women: 85%. Prior to data collection, ethical approval was obtained from the Institutional Ethics Committee at the Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh (India), No. PGI/IEC/2016/2170. The study protocol was also reviewed at the U.S. Centers for Disease Control and Prevention (CDC) and was determined to be a non-research, routine surveillance activity.

2.3 | Informed Consent

Written informed consent (in Hindi) was obtained from all the participants prior to the start of data and blood collection. If the participants were unable to read, the interviewer/literate family member would read out the consent document and the participants could either sign the form or use a thumbprint.

2.4 | Interview Data Collection

All interviews were conducted in the local language (Hindi) at the home of the participants by trained interviewers using structured questionnaires, which were first developed in English and translated into Hindi. The questionnaire had two parts. The household questionnaire collected socio-demographic data and information on participation in the governmental food security public distribution system (PDS), possession of a ration card issued by state governments that enables eligible households to obtain subsidized food from the PDS, and use of wheat grain and flour. Anyone ≥18 years could complete this questionnaire. The questionnaire for women included information on socio-demographics, reproductive and pregnancy history, health conditions, dietary habits, use of nutritional supplements, and medications and was administered to the selected non-pregnant WRA (18–49 years) in the household. Pregnancy status was determined by self-report.

2.5 | Blood Collection, Processing, and Storage

Upon completion of the questionnaire for women, the interviewers described to the participants the blood collection process and provided information on the time and location for blood collection the following day. All blood collections occurred at a central location (e.g., community center/Anganwadi [rural childcare] center/health center or government school). Blood samples were collected from non-pregnant women aged 18–49 years using standardized protocols for laboratory safety and procedures. Women were not required to fast prior to blood collection. Additional details on blood collection, processing, and storage can be found in Appendix.

2.6 | Biochemical Analysis

Biochemical analyses included serum and RBC folate and vitamin B12 concentrations. Serum and RBC folate concentrations were measured using the WHO-recommended microbiologic assay using the chloramphenicol-resistant strain of *Lactobacillus rhamnosus* and a 5-methyltetrahydrofolate calibrator provided by CDC. Serum vitamin B12 concentrations were assessed by the electrochemiluminescence technology-based immunoassay (Cobas e601, Roche analyzer series) using Roche analyzer FDA-approved kits.

2.7 | Biochemical Indicators

Folate deficiency was defined as either serum folate <7 nmol/L or RBC folate <305 nmol/L (Pfeiffer et al. 2016; Institute of Medicine, Food and Nutrition Board 1998; World Health Organization 2015a, 2015b). Folate insufficiency was defined as RBC folate <748 nmol/L, the adjusted threshold for optimal NTD prevention (Pfeiffer et al. 2016, 2011; Crider et al. 2014; Tinker et al. 2015). Vitamin B12 deficiency was defined as vitamin B12 <200 pg/mL, and vitamin B12 marginal deficiency was defined as vitamin B12 ≥200 pg/mL and ≤300 pg/mL (Rosenthal et al. 2017). All women received written reports of their hemoglobin and vitamin B12 levels and beta thalassemia screening results. Women who had severe anemia (hemoglobin <8 g/dL), vitamin B12 deficiency (vitamin B12 <200 pg/mL), or were found to be a carrier for beta thalassemia were contacted by phone and referred to the nearest primary health center.

2.8 | Survey Procedures

Quality assurance procedures were followed for each step of the study. Survey instruments were developed in consultation with experts and were pretested in a similar non-study community. Adequate training including practice interviews were imparted to field investigators for collecting household and women data. Questionnaires were checked for completeness and recording errors by field supervisors at the end of each visit and were returned to interviewers for corrections if necessary. When appropriate, households and women were contacted for follow-up. Laboratory technicians were trained for blood collection, processing, and documentation to ensure good laboratory practices. To ensure accuracy, dual data entry was conducted, and random checks were exercised by field supervisors.

2.9 | Statistical Analysis

Analyses for this descriptive study included non-pregnant, non-lactating women. Frequencies, percentages, and 95% confidence intervals (CIs) were calculated for the following socio-demographic variables: age (18–29, 30–39, 40–49 years), religion (Hinduism, Islam, Sikhism), caste (official governmental classification: General, Scheduled [Caste, Tribe], Other Backward Classes), education (none, primary, secondary, higher secondary and above), occupation (earning, non-earning), marital status (never married, married, divorced/widowed/separated), and parity (nulliparous, primiparous, multiparous). We described the participants' dietary preference as vegetarian, eggetarian (vegetarian who consumes eggs), non-vegetarian, having a functional/active ration card (yes, no), ration card category (below poverty line, above poverty line), PDS wheat purchase (yes, no), folic acid supplement use (yes, no), and vitamin B12 supplement use (yes, no). We calculated, for all variables, the unweighted sample size and weighted percentage and associated 95% CI. All analyses used the study design weights that were adjusted for non-response.

We calculated weighted RBC folate, serum folate, and serum vitamin B12 geometric means (GMs) and GM ratios (GMRs) and 95% CI for all variables using a linear model after natural log transformation. Prevalence estimates and 95% CI for folate deficiency (serum and RBC folate), RBC folate insufficiency, and serum vitamin B12 deficiency and marginal deficiency were calculated. We used a multivariable linear model to calculate the adjusted GMs and GMRs and logistic regression models to calculate the crude and adjusted prevalence ratios (PRs) comparing sub-categories within a variable. For both linear and logistic regression models, we used a stepwise procedure and only retained significant variables ($p < 0.05$) in the final multivariable model. In adjusted models, only parity was included if significant because parity and marital status were correlated.

We modeled the predicted NTD prevalence per 10,000 births based on RBC folate concentrations using the weighted distributions of RBC folate concentrations of the study population (Crider et al. 2014; Wang et al. 2021). All analyses were conducted using SAS-callable SUDAAN Version 11.0.4 (Research Triangle Park, NC).

3 | Results

3.1 | Response Rates

A total of 1155 houses were selected for the survey, and among 1130 eligible households, members from 1115 households (98.7%) participated in the survey. Among the surveyed households, there were 983 eligible non-pregnant WRA (18–49 years), of which 933 (94.9%) agreed to participate in the women's questionnaire and 866 (88.1%) provided blood samples.

3.2 | Characteristics of the Study Population

Blood samples were analyzed from 775 non-pregnant, non-lactating WRA.

Table 1 summarizes the characteristics of these WRA. Their mean age was 33.9 years (data not shown) and 71.1% were 18–39 years old. Among the participants, 88.9% were Hindu; 22.5% had no formal education, 37.8% finished secondary school, 18.0% had higher than secondary education; 68.0% were of a non-earning occupation (homemaker); and 13.6% were never married. Among married women, 82.0% had at least one child. Women were primarily vegetarian (85.4%). Almost all had a ration card (97.1%), and of those with a ration card, 63% were above the poverty line, and 37% were below the poverty line. Most women (65.0%) reported purchasing wheat from PDS fair price shops. Few of the women took folic acid (3.4%) or vitamin B12 (2.5%) supplements.

3.3 | Folate Status

Unadjusted and adjusted geometric means (GMs) and GM ratios (GMRs) of RBC folate concentration are presented in Table 2, and the prevalence of RBC folate deficiency and unadjusted prevalence ratios (PRs) are presented in Table S1. The overall unadjusted and adjusted GMs of RBC folate concentration were 546.3 nmol/L (95% CI: 518.3, 575.8) and 544.5 nmol/L (95% CI: 518.3, 574.8), respectively (Table 2), and 9.3% (95% CI: 7.4, 11.6) of women had RBC folate deficiency (Table S1). RBC folate concentrations were higher in women aged 30–39 years (554.7 nmol/L) and 40–49 years (597.4 nmol/L) compared to women aged 18–29 years (495.1 nmol/L), and differences remained significant after adjustment (aGMR: 1.12 [1.02, 1.23] and aGMR: 1.21 [1.08, 1.35], respectively). Significant differences in GMs were also observed by parity status. Compared to nulliparous women (474.8 nmol/L), women who were primiparous (549.0 nmol/L) and multiparous (566.3 nmol/L) had higher RBC folate concentrations, and differences remained significant after adjustment (aGMR: 1.16 [1.00, 1.33] and aGMR: 1.19 [1.07, 1.33], respectively). Consumption of folic acid or vitamin B12 supplements resulted in higher RBC folate concentrations compared to women who reported no consumption (folic acid: 817.8 vs. 539.1 nmol/L and vitamin B12: 727.2 vs. 542.8 nmol/L), and differences remained significant after adjustment (folic acid: aGMR: 1.52 [1.18, 1.96] and vitamin B12: aGMR:

1.34 [1.00, 1.79]). The overall unadjusted and adjusted GMs of serum folate concentration were identical—12.4 nmol/L (95% CI: 11.9, 12.9; Table S2), and 10.1% (7.9, 12.7) of women had serum folate deficiency (Table S3). Compared to women aged 18–29 years (13.2 nmol/L), serum folate concentration was lower among women aged 40–49 years (11.2 nmol/L, aGMR: 0.85 [0.78, 0.93]). Compared to women with no education (11.9 nmol/L) and those reported not consuming folic acid supplements (12.3 nmol/L), those with higher than a secondary education (13.8 nmol/L, aGMR: 1.16 [1.03, 1.31]) and those who reported consuming folic acid supplements (18.9 nmol/L, aGMR: 1.54 [1.24, 1.91]) had higher serum folate concentrations. Compared to women aged 18–29 years (6.3%) and those who are Hindus (9.6%), serum folate deficiency was higher in women aged 30–39 years (11.0%), women aged 40–49 years (13.0%) and Muslims (28.0%), and differences remained significant after adjustment (30–39 years: aPR: 1.89 [1.02, 3.48]; 40–49 years: aPR: 2.20 [1.14, 4.25]; and Muslims: aPR: 3.29 [1.58, 6.84]).

3.4 | Prevalence of RBC Folate Insufficiency and Predicted NTD Prevalence

The prevalence of RBC folate insufficiency and unadjusted and adjusted prevalence ratios (aPRs) are presented in Table 3. Overall, 78.3% (95% CI: 75.0, 81.3) of women had RBC folate insufficiency. The prevalence of RBC folate insufficiency was 90.2% among women aged 18–29 years and 95.5% among nulliparous women. Women aged 30–39 years (76.6%) and 40–49 years (67.8%) and women who were primiparous (78.6%) and multiparous (74.0%) had lower RBC folate insufficiency compared to women aged 18–29 years and nulliparous women. These differences remained significant after adjustment for women aged 40–49 years and those who were primiparous and multiparous (40–49 years: aPR: 0.84 [0.74, 0.95]; primiparous: aPR: 0.79 [0.67, 0.94]; multiparous: aPR: 0.82 [0.74, 0.90]). Women who reported consuming folic acid supplements had a lower prevalence of folate insufficiency (48.3%) compared to those who reported no consumption (79.4%), and the difference remained after adjustment (aPR: 0.58 [0.34, 0.99]). The overall predicted NTD prevalence per 10,000 births based on RBC folate concentrations was 21.0 (95% uncertainty interval [UI]: 16.9, 25.9).

3.5 | Serum Vitamin B12 Status

The overall unadjusted (190.4 pg/mL [95% CI: 180.6, 200.7]) and adjusted (188.6 pg/mL [178.8, 199.1]) GMs of serum vitamin B12 concentration were similar (Table 4), and 57.7% [53.9, 61.4] of women had serum vitamin B12 deficiency and 23.5% [20.4, 26.9] had marginal deficiency (Table 5). Compared to women aged 18–29 years (167.5 pg/mL), Hindus (183.7 pg/mL), and nulliparous women (152.5 pg/mL), the aGMs of serum vitamin B12 concentration were higher among women aged 40–49 years (224.3 pg/mL, aGMR: 1.34 [1.09, 1.64]), Muslims (274.1 pg/mL, aGMR: 1.49 [1.12, 1.98]) and Sikhs (264.2 pg/mL, aGMR: 1.44 [1.08, 1.92]), and women who were primiparous (224.9 pg/mL, aGMR: 1.47 [1.17, 1.85]) and multiparous (198.2 pg/mL, aGMR: 1.30 [1.09, 1.54]). Compared to women who reported no consumption of folic acid supplements (188.9 pg/mL) or vitamin B12 supplements (187.0 pg/mL), the aGMs of serum vitamin B12 concentration were higher among women who reported consuming folic acid supplements (278.4 pg/mL, aGMR: 1.47 [1.04, 2.09]) or vitamin B12 supplements (468.3 pg/mL, aGMR: 2.50 [1.51, 4.15]). Prevalence of serum vitamin B12 deficiency was lower among women aged 40–49 years

(48.8%) compared to women aged 18–29 years (69.3%, aPR: 0.80 [0.64, 0.99]); Muslims (36.9%) and Sikhs (38.4%) compared to Hindus (60.2%, Muslims: aPR: 0.55 [0.30, 1.00] and Sikhs: aPR: 0.67 [0.49, 0.90]); multiparous women (53.1%) compared to nulliparous women (75.6%, aPR: 0.81 [0.65, 0.99]); and those who reported consuming vitamin B12 supplements (16.8%) compared to those who reported no consumption of vitamin B12 supplements (58.8%, aPR: 0.29 [0.12, 0.70]).

4 | Discussion

This is the first population-based survey to provide blood folate and vitamin B12 status among non-pregnant, non-lactating WRA in Ambala District, Haryana, India. The prevalence of serum and RBC folate deficiency were 10.1% and 9.3%, respectively. Almost 80% of WRA had RBC folate insufficiency (RBC folate concentrations below the WHO threshold for optimal NTD prevention), 57.7% had vitamin B12 deficiency, and 23.5% had vitamin B12 marginal deficiency.

RBC folate insufficiency is associated with increased risk for NTDs at a population level (Crider et al. 2014). The prevalence of RBC folate insufficiency found in our study was very similar to the prevalence reported in a study from South India (79.3%) (Finkelstein et al. 2021). The method used to assess RBC folate insufficiency in these two studies was very similar. Both studies used the same WHO recommended microbiologic assay method, the same cut-off (<748 nmol/L for RBC folate insufficiency), and the same exclusion of pregnant and lactating women. Although several countries assessed RBC folate status in WRA, only few countries (United States, Belize, Guatemala, Ireland, New Zealand, and the United Kingdom) used the same microbiologic assay method and corresponding cut-off values as those used in the two Indian surveys (Pfeiffer et al. 2019) enabling comparable comparisons. The prevalence of RBC folate insufficiency reported in our study as well as in the study from South India were higher compared to those reported in the United States (18.6% in 2011–2016), Guatemala (47.2% in 2009–2010), Belize (48.9% in 2011), Ireland (64% in 2008–2010), and New Zealand (73% in 2008–2009) (Pfeiffer et al. 2019; Rosenthal et al. 2016, 2017; Hopkins et al. 2015; University of Otago 2011). In the United States, the prevalence was assessed post mandatory folic acid fortification while folic acid fortification was voluntary in the other four countries when the prevalence was assessed. There was no folic acid fortification in the United Kingdom at the time of assessment and the prevalence of RBC folate insufficiency was reported to be 91% (Public Health England 2018). One region in Guatemala was found to also have a high prevalence of RBC folate insufficiency (88.8%) and was thought to have limited access to fortified staples (Rosenthal et al. 2016). In our study, the prevalence of RBC folate insufficiency varied by age, parity, and folic acid supplement use. The prevalence was higher among WRA who were younger, nulliparous, and not reported to have used folic acid supplements; these findings are consistent with previously reported findings in South India (Finkelstein et al. 2021).

Using the RBC folate distribution, the predicted NTD prevalence in our study population (21.0 per 10,000 live births) was very similar to the estimated NTD prevalence found in South India where folic acid fortification program had not been implemented (20.6 per 10,000 live births) (Finkelstein et al. 2021). However, our estimated NTD prevalence is lower than the national pooled estimates. Comparability is challenging since these estimates relied on data using varying case definitions, types of hospitals, and time periods. A region in Guatemala where the population was thought to have limited access to fortified staples reported an even higher estimated NTD prevalence (26 per 10,000 live births) (Rosenthal et al. 2016). The estimated NTD prevalence in our study population was much higher than in countries with mandatory folic acid fortification, for example, 7.3 per 10,000 live births in the United States and 11 per 10,000 live births in the Metropolitan region of Guatemala where the population likely had access to fortified wheat flour (Crider et al. 2018; Rosenthal et al. 2016).

Serum folate is supposed to reflect recent intake, while RBC folate reflects intake over 120 days. In our study, the prevalence of folate deficiency was similar in the two media (10.1% in serum and 9.3% in RBC). Different methods and cut-offs are used for folate deficiency globally, making comparisons of estimates challenging. However, the study in South India did use the same methodology and cut-offs and serum and RBC folate deficiency were both higher in our study compared to findings from South India (serum folate deficiency 3.5% and RBC folate deficiency 7.6%) (Finkelstein et al. 2021). RBC folate deficiency in our study did not vary significantly among different characteristics. Studies in both China (Chen et al. 2019>) and South India (Fothergill et al. 2023) highlight that a serum folate insufficiency threshold should not be used to estimate NTD prevalence in populations with vitamin B12 deficiency and insufficiency. Thus, serum folate insufficiency was not used to estimate NTD prevalence in our study.

The prevalence of vitamin B12 deficiency in our study (57.7%) is one of the highest reported among WRA globally and was comparable to findings from other studies in India (42% in rural areas in Nagpur district, Maharashtra; 45% in rural areas in Mahbubnagar district, Telangana; 48.3% in South India; 50% in Pune, Maharashtra) and Pakistan (52.4% from a national survey) (Yajnik et al. 2008; Barney et al. 2020; Menon et al. 2011; Singh et al. 2017; Finkelstein et al. 2021; Naik, Mahalle, and Bhide 2018; Soofi et al. 2017). The prevalence of vitamin B12 deficiency in our study was higher among WRA who were younger, Hindus, nulliparous, and not reported to have used vitamin B12 supplements. These findings are consistent with findings from other studies (Sivaprasad et al. 2016; Finkelstein et al. 2021). Although women who reported to be vegetarians in our study had a higher prevalence of vitamin B12 deficiency (58.8%), it was not significantly higher than those who reported to be eggetarians (51.8%) and non-vegetarians (50.8%), who also appeared to have high prevalence of vitamin B12 deficiency. Although it was surprising to see the high prevalence of vitamin B12 deficiency among eggetarians and non-vegetarians in our study, this could be associated with the low frequency of animal-source foods (ASFs) consumption in the eggetarians and non-vegetarians, as low ASFs consumption had been identified as a risk factor for vitamin B12 deficiency (Green et al. 2017). This is also seen in other studies in India where a high prevalence of vitamin B12 insufficiency was reported among non-vegetarians (Finkelstein et al. 2021). In addition, a self-reported vegetarian diet had previously been reported to not be significantly associated with lower vitamin B12 concentrations (Menon et al. 2011). Women who were Hindus in our study had a significantly higher prevalence of vitamin B12 deficiency (60.2%) compared to women who were Muslims (36.9%) and Sikhs (38.4%). Different religious dietary rules have been reported to be risk factors for nutritional inadequacies or malnutrition, particularly in populations who consume no meat and/or poor dairy product intake (Chouraqui et al. 2021). The prevalence of vitamin B12 marginal deficiency in our study was 23.5%; hence vitamin B12 insufficiency was 81.2% (deficiency+marginal deficiency).

Our findings of the high baseline burden of RBC folate insufficiency and vitamin B12 deficiency/insufficiency suggest fortification with folic acid and vitamin B12 could reduce the prevalence of these micronutrient insufficiencies/deficiencies as had been observed in other countries (Das et al. 2013; Cordero, Do, and Berry 2008; López-Camelo et al. 2005; Sayed et al. 2008; Calvo and Biglieri 2008; Williams et al. 2002; Liu et al. 2004; Persad et al. 2002; De Wals et al. 2003; Ray et al. 2002). Given that the consumption of wheat in Haryana was >300 g/per capita/day, opportunities exist to fortify wheat flour to increase the intake of folic acid, vitamin B12, and other micronutrients among the populations in Haryana (Food Fortification Initiative 2016).

Our study is the first to provide representative population-based estimates on folate and vitamin B12 status among non-pregnant, non-lactating WRA in the rural Ambala District, Haryana. These results will serve as a baseline against which results from future post-fortification surveys can be compared. It is also the first study in North India to use distributions of RBC folate concentrations as measured by the microbiologic assay among WRA in rural Ambala District to estimate the predicted NTD prevalence in the population. Predicted prevalence of NTDs using RBC folate concentrations is valuable because there are presently no published studies on actual population estimates of NTDs in the study area. Our study was designed and implemented using a reliable population-based sampling design to reduce biases in the selection of households and individuals. Strict field and laboratory methodologies and rigorous protocols were used for survey administration and to ensure proper handling of biological samples. Folate analysis was performed using the WHO recommended microbiologic assay which enabled us to compare our findings with other studies that also used the same assay. Participation rates for both questionnaire and blood sample collection were high in our study.

Our study has several limitations. The cross-sectional survey design could not be used to determine causation. Because dietary intake data were not collected on folic acid or vitamin B12, we are unable to assess the causes of some of the disparities in observed deficiency and insufficiency. Our study only measured serum vitamin B12 status and other forms of vitamin B12 (e.g., methylmalonic acid, holo-transcobalamin) were not assessed.

In conclusion, this population-based cross-sectional study provides baseline pre-fortification measures of folate and vitamin B12 status among non-pregnant, non-lactating WRA in Haryana, India against which results from future post -fortification surveys can be compared. In our study, almost 80% of non-pregnant, non-lactating women in rural Ambala District, Haryana had RBC folate insufficiency with an estimated NTD prevalence of 21 per 10,000 live births. In addition, almost 60% of WRA had vitamin B12 deficiency, and 80% had vitamin B12 insufficiency.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix

Blood Collection, Processing, and Storage

For each participant, venous blood samples (12 mL) were collected in two EDTA coated vacutainer and one non-coated vacutainer for serum separation. The collected blood samples were stored immediately at optimal cold chain (between 4 and 8°C) to ensure minimal sample degradation during transport to either a regional hospital laboratory for processing or directly to the PGIMER Department of Hematology Laboratory in Chandigarh. Samples processed at the regional hospital laboratory were transported to the PGIMER Department of Hematology Laboratory in Chandigarh in portable freezers twice a week at −20°C. Sample processing included preparing aliquots of hemolysates in freshly prepared ascorbic acid as per CDC protocol for the microbiologic assay for red blood cell (RBC) folate assessment, separation of serum, and aliquoting samples. All sample aliquots awaiting transport to PGIMER were stored at −20°C. Precautions were taken to ensure that the blood samples were not exposed to bright light. Complete hemogram was performed using the hematology analyzer—Sysmex XP 100 for hemoglobin (Hb) estimation and automated red cell indices. Hematocrit obtained from the hemogram was used to calculate the final RBC folate concentration. Once at PGIMER, the samples were stored at −80°C or less until batch analysis.

Biochemical Analysis

Both internal quality control as well as external quality assessment scheme from CDC (CDC VITAL-EQA) were used to ensure the quality of the RBC and serum folate results. CDC provided logistical support, and internal quality control (QC) pools were set up at PGIMER before performing analyses on the study samples (Das et al. 2017). QC pools for low and high whole-blood and serum folate concentrations were prepared and aliquots stored at

−80°C. For each run, a standard growth curve of the organisms was prepared. Four replicates were run for each individual sample to calculate a robust mean concentration. The growth of the organisms was read as turbidity after incubating at 37°C for 40–42 h. The intra- and inter- assay coefficients of variation (CVs) for the high serum folate QC were 7.1% and 7.4%, respectively and for the low serum folate QC were 6.3% and 9.4%, respectively. The intra- and inter-assay CVs for the high whole blood lysate (WBL) folate QC were 6.8% and 7.7%, respectively and for the low WBL folate QC were 6.7% and 9.8%, respectively. The intra- and inter-assay CVs for the high serum vitamin B12 QC were 2.3% and 5.4%, respectively and for the low serum vitamin B12 QC were 1.9% and 7.4%, respectively.

Data Availability Statement

Research data are not shared.

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TABLE 1 |

Population characteristics among non-pregnant and non-lactating women of reproductive age, 18–49 years, Ambala District, Haryana, India.

Characteristic	*n* (unweighted)	Weighted (%)	95% CI
Total	775		
Age (years)			
18–29	249	31.3	28.0, 34.9
30–39	307	39.8	36.1, 43.6
40–49	219	28.9	25.5, 32.5
Religion			
Hinduism	693	88.9	86.6, 90.8
Islam	22	2.9	1.9, 4.4
Sikhism	60	8.2	6.5, 10.3
Caste			
General	229	29.1	26.2, 32.1
Scheduled (caste, tribe)	279	33.2	30.4, 36.2
Other backward classes	267	37.7	34.8, 40.7
Education			
None	160	22.5	19.3, 26.0
Primary	155	21.7	18.7, 25.1
Secondary	291	37.8	34.1, 41.6
Higher secondary and above	137	18.0	15.3, 21.0
Missing	32		
Occupation			
Earning	248	32.0	28.5, 35.7
Non-earning	513	68.0	64.3, 71.5
Missing	14		
Marital status			
Never married	109	13.6	11.2, 16.3
Married	620	79.8	76.5, 82.7
Divorced/widowed/separated	46	6.6	4.9, 8.9
Parity			

Characteristic	***n* (unweighted)**	**Weighted (%)**	**95% CI**
Nulliparous	145	18.0	15.4, 21.1
Primiparous	79	10.1	8.0, 12.6
Multiparous	551	71.9	68.4, 75.2
Diet			
Vegetarian	670	85.4	82.4, 88.0
Eggetarian	36	4.7	3.4, 6.6
Non-vegetarian	69	9.8	7.7, 12.5
Having a ration card			
Yes	755	97.1	95.3, 98.2
No	20	2.9	1.8, 4.7
Ration card category			
Below poverty line	277	36.2	32.8, 39.7
Above poverty line	477	60.9	57.2, 64.4
No ration card	20	2.9	1.8, 4.7
Missing	1		
PDS wheat purchase			
Yes	497	65.0	61.4, 68.4
No	255	32.1	28.8, 35.6
No ration card	20	2.9	1.8, 4.7
Missing	3		
Folic acid supplement use			
Yes	23	3.4	2.2, 5.3
No	752	96.6	94.7, 97.8
Vitamin B12 supplement use			
Yes	20	2.5	1.6, 4.0
No	755	97.5	96.0, 98.4

Abbreviations: 95% CI, 95% confidence interval; PDS, public distribution system.

TABLE 2 |

Unadjusted and adjusted geometric means and geometric mean ratios of red blood cell folate concentrations among non-pregnant and non-lactating women of reproductive age, 18–49 years, Ambala District, Haryana, India.

		RBC folate, nmol/L			
Characteristic	***n* (unweighted)**	**Unadjusted geometric mean (95% CI)**	**Unadjusted geometric mean ratio (95% CI)**	**Adjusted geometric mean (95% CI)[a]**	**Adjusted geometric mean ratio (95% CI)**
Total	775	546.3 (518.3, 575.8)		544.5 (518.3, 574.8)	
Age (years)					
18–29	249	464.9 (438.9, 492.4)	Referent	495.1 (463.4, 528.9)	Referent
30–39	307	566.7 (535.5, 599.7)	**1.22 (1.12, 1.32)**	554.7 (522.4, 588.9)	**1.12 (1.02, 1.23)**
40–49	219	621.0 (576.8, 668.6)	**1.34 (1.22, 1.47)**	597.4 (554.6, 643.6)	**1.21 (1.08, 1.35)**
Religion					
Hinduism	693	543.1 (523.2, 563.8)	Referent		
Islam	22	514.6 (411.9, 643.1)	0.95 (0.76, 1.19)		
Sikhism	60	601.5 (514.5, 703.2)	1.11 (0.94, 1.30)		
Caste					
General	229	567.5 (529.2, 608.7)	Referent		
Scheduled (caste, tribe)	279	542.2 (507.2, 579.6)	0.96 (0.87, 1.05)		
Other backward classes	267	535.4 (506.8, 565.7)	0.94 (0.86, 1.03)		
Education					
None	160	546.5 (509.1, 586.7)	Referent		
Primary	155	564.3 (521.3, 610.9)	1.03 (0.93, 1.15)		
Secondary	291	530.1 (497.7, 564.6)	0.97 (0.88, 1.07)		
Higher secondary and above	137	549.8 (504.0, 599.7)	1.01 (0.90, 1.13)		
Occupation					
Earning	248	547.3 (512.6, 584.4)	Referent		
Non-earning	513	544.0 (520.4, 568.6)	0.99 (0.92, 1.08)		
Marital status					
Never married	109	427.9 (388.3, 471.4)	Referent		
Married	620	570.4 (547.9, 593.7)	**1.33 (1.20, 1.48)**		
Divorced/widowed/separated	46	545.1 (483.3, 614.7)	**1.27 (1.09, 1.49)**		
Parity					

Characteristic	*n* (unweighted)	RBC folate, nmol/L Unadjusted geometric mean (95% CI)	Unadjusted geometric mean ratio (95% CI)	Adjusted geometric mean (95% CI)[a]	Adjusted geometric mean ratio (95% CI)
Nulliparous	145	434.3 (401.2, 470.2)	Referent	474.8 (431.9, 522.0)	Referent
Primiparous	79	521.0 (465.0, 583.8)	**1.20 (1.04, 1.38)**	549.0 (489.9, 615.2)	**1.16 (1.00 [b], 1.33)**
Multiparous	551	583.3 (558.9, 608.8)	**1.34 (1.23, 1.47)**	566.3 (543.0, 590.5)	**1.19 (1.07, 1.33)**
Diet					
Vegetarian	670	541.9 (520.9, 563.7)	Referent		
Eggetarian	36	519.0 (461.8, 583.2)	0.96 (0.85, 1.08)		
Non-vegetarian	69	607.3 (541.4, 681.2)	1.12 (0.99, 1.27)		
Having a ration card					
Yes	755	547.4 (527.9, 567.7)	1.04 (0.85, 1.26)		
No	20	527.8 (434.7, 640.8)	Referent		
Ration card category					
Below poverty line	277	532.5 (502.8, 563.9)	Referent		
Above poverty line	477	556.5 (530.3, 584.0)	1.05 (0.97, 1.13)		
No ration card	20	527.8 (434.7, 640.8)	0.99 (0.81, 1.21)		
PDS wheat purchase					
Yes	497	539.5 (516.3, 563.7)	1.02 (0.84, 1.25)		
No	255	564.7 (528.4, 603.5)	1.07 (0.87, 1.32)		
No ration card	20	527.8 (434.7, 640.8)	Referent		
Folic acid supplement use					
Yes	23	863.6 (685.4, 1088.2)	**1.61 (1.27, 2.03)**	817.8 (637.4, 1049.4)	**1.52 (1.18, 1.96)**
No	752	538.1 (519.3, 557.5)	Referent	539.1 (520.8, 558.0)	Referent
Vitamin B12 supplement use					
Yes	20	835.1 (623.6, 1118.5)	**1.54 (1.15, 2.07)**	727.2 (547.2, 966.3)	**1.34 (1.00 [b], 1.79)**
No	755	540.8 (522.0, 560.3)	Referent	542.8 (524.6, 561.6)	Referent

Note: Statistically significant results are bolded.

Abbreviations: 95% CI, 95% confidence interval; PDS, public distribution system; RBC, red blood cell.

[a]Adjusted for age, parity, folic acid use, and vitamin B12 use.

[b]Value >1.

TABLE 3 |

Prevalence and unadjusted and adjusted prevalence ratios of red blood cell folate insufficiency among non-pregnant and non-lactating women of reproductive age, 18–49 years, Ambala District, Haryana, India.

		RBC folate insufficiency (<748 nmol/L)		
Characteristic	***n* (unweighted)**	**Prevalence (95% CI)**	**Unadjusted prevalence ratio (95% CI)**	**Adjusted prevalence ratio (95% CI)[a]**
Total	775	78.3 (75.0, 81.3)		
Age (years)				
18–29	249	90.2 (85.0, 93.7)	Referent	Referent
30–39	307	76.6 (71.0, 81.5)	**0.85 (0.78, 0.92)**	0.92 (0.83, 1.02)
40–49	219	67.8 (60.6, 74.3)	**0.75 (0.67, 0.84)**	**0.84 (0.74, 0.95)**
Religion				
Hinduism	693	79.1 (75.7, 82.2)	Referent	
Islam	22	77.2 (53.9, 90.7)	0.98 (0.76, 1.25)	
Sikhism	60	70.2 (54.8, 82.1)	0.89 (0.72, 1.09)	
Caste				
General	229	75.3 (68.5, 81.0)	Referent	
Scheduled (caste, tribe)	279	77.4 (71.6, 82.3)	1.03 (0.92, 1.15)	
Other backward classes	267	81.6 (75.7, 86.2)	1.08 (0.97, 1.20)	
Education				
None	160	78.9 (70.9, 85.2)	Referent	
Primary	155	78.0 (70.0, 84.4)	0.99 (0.87, 1.13)	
Secondary	291	79.7 (74.3, 84.2)	1.01 (0.90, 1.13)	
Higher secondary and above	137	78.2 (69.7, 84.8)	0.99 (0.87, 1.13)	
Occupation				
Earning	248	77.1 (70.9, 82.4)	Referent	
Non-earning	513	79.1 (74.9, 82.8)	1.03 (0.94, 1.12)	
Marital status				
Never married	109	94.7 (85.3, 98.2)	Referent	
Married	620	75.7 (71.8, 79.2)	**0.80 (0.74, 0.86)**	
Divorced/widowed/separated	46	76.3 (61.6, 86.6)	**0.81 (0.68, 0.96)**	
Parity				

		RBC folate insufficiency (<748 nmol/L)		
Characteristic	***n* (unweighted)**	**Prevalence (95% CI)**	**Unadjusted prevalence ratio (95% CI)**	**Adjusted prevalence ratio (95% CI)[a]**
Nulliparous	145	95.5 (88.5, 98.4)	Referent	Referent
Primiparous	79	78.6 (66.1, 87.4)	**0.82 (0.71, 0.95)**	**0.79 (0.67, 0.94)**
Multiparous	551	74.0 (69.8, 77.8)	**0.77 (0.72, 0.83)**	**0.82 (0.74, 0.90)**
Diet				
Vegetarian	670	78.8 (75.3, 82.0)	Referent	
Eggetarian	36	87.5 (72.4, 95.0)	1.11 (0.98 1.26)	
Non-vegetarian	69	69.8 (56.5, 80.4)	0.88 (0.74, 1.06)	
Having a ration card				
Yes	755	78.1 (74.7, 81.1)	0.89 (0.76, 1.05)	
No	20	87.6 (67.2, 96.0)	Referent	
Ration card category				
Below poverty line	277	80.4 (74.9, 85.0)	Referent	
Above poverty line	477	76.6 (72.1, 80.6)	0.95 (0.88, 1.04)	
No ration card	20	87.6 (67.2, 96.0)	1.09 (0.92, 1.29)	
PDS wheat purchase				
Yes	497	79.8 (75.7, 83.3)	0.91 (0.78, 1.07)	
No	255	74.7 (68.1, 80.3)	0.85 (0.72, 1.02)	
No ration card	20	87.6 (67.2, 96.0)	Referent	
Folic acid supplement use				
Yes	23	48.3 (27.5, 69.6)	**0.61 (0.38, 0.97)**	**0.58 (0.34, 0.99)**
No	752	79.4 (76.1, 82.4)	Referent	Referent
Vitamin B12 supplement use				
Yes	20	62.7 (37.7, 82.3)	0.80 (0.54, 1.17)	
No	755	78.8 (75.4, 81.8)	Referent	

Note: Statistically significant results are bolded.

Abbreviations: 95% CI, 95% confidence interval; PDS, public distribution system; RBC, red blood cell.

[a]Adjusted for age, parity, and folic acid use.

TABLE 4 |

Unadjusted and adjusted geometric means and geometric mean ratios of serum vitamin B12 concentrations among non-pregnant and non-lactating women of reproductive age, 18–49 years, Ambala District, Haryana, India.

		Serum vitamin B12, pg/dL			
Characteristic	***n* (unweighted)**	**Unadjusted geometric mean (95% CI)**	**Unadjusted geometric mean ratio (95% CI)**	**Adjusted geometric mean (95% CI)[a]**	**Adjusted geometric mean ratio (95% CI)**
Total	755	190.4 (180.6, 200.7)		188.6 (178.8, 199.1)	
Age (years)					
18–29	249	154.5 (142.3, 167.8)	Referent	167.5 (150.7, 186.2)	Referent
30–39	307	193.7 (176.9, 212.1)	1.25 (1.11, 1.42)	189.6 (173.0, 207.8)	1.13 (0.97, 1.32)
40–49	219	237.7 (206.3, 273.9)	**1.54 (1.30, 1.81)**	224.3 (194.7, 258.4)	**1.34 (1.09, 1.64)**
Religion					
Hinduism	693	183.5 (172.8, 194.9)	Referent	183.7 (173.7, 194.2)	Referent
Islam	22	248.4 (186.7, 330.4)	**1.35 (1.01, 1.81)**	274.1 (207.6, 361.8)	**1.49 (1.12, 1.98)**
Sikhism	60	276.5 (202.0, 378.4)	**1.51 (1.09, 2.08)**	264.2 (199.6, 349.7)	**1.44 (1.08, 1.92)**
Caste					
General	229	189.3 (170.1, 210.7)	Referent		
Scheduled (caste, tribe)	279	188.5 (167.7, 212.0)	1.00 (0.85, 1.18)		
Other backward classes	267	196.5 (178.8, 216.0)	1.04 (0.90, 1.21)		
Education					
None	160	200.9 (176.8, 228.3)	Referent		
Primary	155	193.2 (169.6, 220.1)	0.96 (0.80, 1.15)		
Secondary	291	187.2 (169.0, 207.3)	0.93 (0.79, 1.10)		
Higher secondary and above	137	189.4 (159.0, 225.5)	0.94 (0.76, 1.17)		
Occupation					
Earning	248	197.1 (175.2, 221.8)	Referent		
Non-Earning	513	189.3 (176.0, 203.6)	0.96 (0.83, 1.11)		
Marital status					
Never married	109	135.1 (121.0, 150.8)	Referent		
Married	620	202.6 (188.7, 217.5)	**1.50 (1.31, 1.72)**		
Divorced/widowed/separated	46	197.9 (157.2, 249.1)	**1.47 (1.13, 1.89)**		
Parity					

Characteristic	*n* (unweighted)	Serum vitamin B12, pg/dL: Unadjusted geometric mean (95% CI)	Unadjusted geometric mean ratio (95% CI)	Adjusted geometric mean (95% CI)[a]	Adjusted geometric mean ratio (95% CI)
Nulliparous	145	132.9 (119.6, 147.8)	Referent	152.5 (132.5, 175.5)	Referent
Primiparous	79	210.0 (160.6, 274.6)	**1.58 (1.18, 2.12)**	224.9 (175.5, 288.1)	**1.47 (1.17, 1.85)**
Multiparous	551	207.1 (192.9, 222.3)	**1.56 (1.37, 1.78)**	198.2 (184.7, 212.6)	**1.30 (1.09, 1.54)**
Diet					
Vegetarian	670	187.0 (175.5, 199.2)	Referent		
Eggetarian	36	178.5 (142.8, 223.1)	0.95 (0.76, 1.21)		
Non-vegetarian	69	243.2 (186.7, 316.7)	1.30 (0.99, 1.71)		
Having a ration card					
Yes	755	191.2 (179.8, 203.2)	0.95 (0.77, 1.17)		
No	20	201.5 (164.6, 246.7)	Referent		
Ration card category					
Below poverty line	277	183.4 (169.0, 199.0)	Referent		
Above poverty line	477	195.9 (179.5, 213.7)	1.07 (0.95, 1.21)		
No ration card	20	201.5 (164.6, 246.7)	1.10 (0.89, 1.36)		
PDS wheat purchase					
Yes	497	184.6 (172.0, 198.1)	0.92 (0.74, 1.13)		
No	255	205.1 (180.8, 232.7)	1.02 (0.80, 1.29)		
No ration card	20	201.5 (164.6, 246.7)	Referent		
Folic acid supplement use					
Yes	23	331.5 (205.8, 534.0)	**1.77 (1.09, 2.85)**	278.4 (197.6, 392.2)	**1.47 (1.04, 2.09)**
No	752	187.8 (177.0, 199.2)	Referent	188.9 (178.7, 199.8)	Referent
Vitamin B12 supplement use					
Yes	20	525.6 (295.5, 934.9)	**2.82 (1.58, 5.04)**	468.3 (283.4, 773.8)	**2.50 (1.51, 4.15)**
No	755	186.5 (176.0, 197.6)	Referent	187.0 (177.0, 197.6)	Referent

Note: Statistically significant results are bolded.

Abbreviations: 95% CI, 95% confidence interval; PDS, public distribution system.

[a] Adjusted for: Age, religion, parity, folic acid use, and vitamin B12 use.

TABLE 5 |

Prevalence and unadjusted and adjusted prevalence ratios of vitamin B12 deficiency and marginal deficiency among non-pregnant and non-lactating women of reproductive age, 18–49 years, Ambala District, Haryana, India.

		Serum vitamin B12 deficiency (<200 pg/mL)			Serum vitamin B12 marginal deficiency (≥200–<300 pg/mL)		
Characteristic	***n* (unweighted)**	**Prevalence (95% CI)**	**Unadjusted prevalence ratio (95% CI)**	**Adjusted prevalence ratio (95% CI)[a]**	**Prevalence (95% CI)**	**Unadjusted prevalence ratio (95% CI)**	**Adjusted prevalence ratio (95% CI)[b]**
Total	755	57.7 (53.9, 61.4)			23.5 (20.4, 26.9)		
Age (years)							
18–29	249	69.3 (62.8, 75.1)	Referent	Referent	18.8 (14.2, 24.4)	Referent	Referent
30–39	307	55.1 (49.1, 60.9)	**0.79 (0.69, 0.91)**	0.86 (0.71, 1.05)	26.6 (21.5, 32.3)	**1.42 (1.01, 1.99)**	1.05 (0.68, 1.63)
40–49	219	48.8 (41.5, 56.1)	**0.70 (0.59, 0.84)**	**0.80 (0.64, 0.99)**	24.5 (18.8, 31.4)	1.31 (0.90, 1.90)	0.96 (0.60, 1.54)
Religion							
Hinduism	693	60.2 (56.2, 64.0)	Referent	Referent	21.8 (18.6, 25.4)	Referent	Referent
Islam	22	36.9 (19.5, 58.6)	0.61 (0.35, 1.07)	**0.55 (0.30, 1.00 [c])**	37.8 (19.5, 60.4)	1.73 (0.96, 3.13)	1.64 (0.86, 3.13)
Sikhism	60	38.4 (26.7, 51.6)	**0.64 (0.45, 0.89)**	**0.67 (0.49, 0.90)**	36.9 (24.4, 51.5)	**1.69 (1.13, 2.54)**	**1.57 (1.04, 2.37)**
Caste							
General	229	54.1 (47.1, 60.9)	Referent		28.6 (22.6, 35.4)	Referent	
Scheduled (caste, tribe)	279	57.3 (51.0, 63.4)	1.06 (0.90, 1.26)		21.7 (16.8, 27.4)	0.76 (0.54, 1.05)	
Other backward classes	267	60.8 (54.2, 67.0)	1.12 (0.95, 1.33)		21.3 (16.3, 27.3)	0.74 (0.53, 1.05)	
Education							
None	160	52.6 (44.3, 60.8)	Referent		32.5 (25.0, 41.1)	Referent	Referent
Primary	155	61.7 (53.2, 69.6)	1.17 (0.95, 1.44)		19.5 (13.5, 27.2)	**0.60 (0.39, 0.92)**	**0.60 (0.39, 0.92)**
Secondary	291	57.4 (51.1, 63.4)	1.09 (0.90, 1.32)		21.3 (16.8, 26.8)	**0.66 (0.47, 0.92)**	**0.66 (0.47, 0.93)**
Higher secondary and above	137	59.8 (50.9, 68.2)	1.14 (0.92, 1.41)		22.0 (15.5, 30.1)	0.68 (0.44, 1.03)	0.69 (0.45, 1.04)
Occupation							
Earning	248	54.9 (48.1, 61.5)	Referent		25.8 (20.2, 32.2)	Referent	
Non-earning	513	58.9 (54.2, 63.4)	1.07 (0.93, 1.24)		22.7 (19.0, 27.0)	0.88 (0.66, 1.19)	
Marital status							
Never married	109	78.7 (68.7, 86.1)	Referent		12.5 (7.2, 20.8)	Referent	
Married	620	54.0 (49.7, 58.2)	**0.69 (0.60, 0.79)**		25.7 (22.1, 29.7)	**2.06 (1.19, 3.57)**	

Characteristic	*n* (unweighted)	Serum vitamin B12 deficiency (<200 pg/mL) Prevalence (95% CI)	Unadjusted prevalence ratio (95% CI)	Adjusted prevalence ratio (95% CI)[a]	Serum vitamin B12 marginal deficiency (≥200–<300 pg/mL) Prevalence (95% CI)	Unadjusted prevalence ratio (95% CI)	Adjusted prevalence ratio (95% CI)[b]
Divorced/widowed/separated	46	59.5 (44.0, 73.4)	**0.76 (0.57, 1.00**[c])		20.2 (10.8, 34.7)	1.62 (0.74, 3.56)	
Parity							
Nulliparous	145	75.6 (67.0, 82.6)	Referent	Referent	14.7 (9.3, 22.5)	Referent	Referent
Primiparous	79	58.4 (46.1, 69.7)	**0.77 (0.61, 0.97)**	0.81 (0.63, 1.04)	22.9 (14.5, 34.2)	1.55 (0.84, 2.88)	1.47 (0.79, 2.73)
Multiparous	551	53.1 (48.6, 57.6)	**0.70 (0.61, 0.80)**	**0.81 (0.65, 0.99)**	25.8 (22.0, 30.1)	**1.75 (1.10, 2.80)**	**1.70 (1.08, 2.68)**
Diet							
Vegetarian	670	58.8 (54.8, 62.8)	Referent		23.1 (19.7, 26.7)	Referent	
Eggetarian	36	51.8 (34.6, 68.7)	0.88 (0.62, 1.25)		37.3 (21.8, 55.9)	1.62 (0.98, 2.66)	
Non-vegetarian	69	50.8 (38.0, 63.5)	0.86 (0.66, 1.13)		21.1 (12.4, 33.5)	0.91 (0.54, 1.55)	
Having a ration card							
Yes	755	58.0 (54.2, 61.7)	1.20 (0.73, 1.96)		23.2 (20.0, 26.6)	0.64 (0.33, 1.27)	
No	20	48.5 (26.7, 70.9)	Referent		36.0 (16.6, 61.4)	Referent	
Ration card category							
Below poverty line	277	58.2 (51.8, 64.3)	Referent		24.3 (19.2, 30.2)	Referent	
Above poverty line	477	57.8 (52.9, 62.5)	0.99 (0.87, 1.14)		22.5 (18.7, 26.9)	0.93 (0.69, 1.24)	
No ration card	20	48.5 (26.7, 70.9)	0.83 (0.50, 1.38)		36.0 (16.6, 61.4)	1.48 (0.73, 3.00)	
PDS wheat purchase							
Yes	497	59.4 (54.7, 63.9)	1.22 (0.75, 2.01)		23.0 (19.2, 27.3)	0.64 (0.32, 1.27)	
No	255	55.4 (48.5, 62.0)	1.14 (0.69, 1.89)		23.0 (17.8, 29.2)	0.64 (0.32, 1.29)	
No ration card	20	48.5 (26.7, 70.9)	Referent		36.0 (16.6, 61.4)	Referent	
Folic acid supplement use							
Yes	23	33.8 (17.5, 55.3)	0.58 (0.32, 1.04)		14.7 (4.5, 38.6)	0.62 (0.20, 1.87)	
No	752	58.6 (54.7, 62.3)	Referent		23.8 (20.7, 27.3)	Referent	
Vitamin B12 supplement use							
Yes	20	16.8 (6.5, 37.0)	**0.29 (0.12, 0.70)**	**0.29 (0.12, 0.70)**	15.6 (5.6, 36.5)	0.66 (0.25, 1.72)	
No	755	58.8 (55.0, 62.5)	Referent	Referent	23.7 (20.6, 27.2)	Referent	

Note: Statistically significant results are bolded.

Abbreviations: 95% CI, 95% confidence interval; PDS, public distribution system.

[a]Adjusted for age, religion, parity, and vitamin B12 use.

[b]Adjusted for religion, education, and parity.

[c]Value >1.