ORIGINAL RESEARCH

CURRENT DEVELOPMENTS IN NUTRITION

Community and Global Nutrition

Anemia and Vitamin B-12 and Folate Status in Women of Reproductive Age in Southern India: Estimating Population-Based Risk of Neural Tube Defects

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ABSTRACT

Background: Women of reproductive age (WRA) are a high-risk population for anemia and micronutrient deficiencies. However, there are few representative population-level data from India, which could help inform evidence-based recommendations and policy.

Objective: To conduct a population-based biomarker survey of anemia and vitamin B-12 and folate status in WRA as part of a periconceptional surveillance program in southern India.

Methods: Participants were WRA (15–40 y) who were not pregnant or lactating. Whole blood ($n = 979$) was analyzed for hemoglobin via a Coulter counter (Coulter HMX). Plasma, serum, and RBCs were processed and stored at −80°C or less until batch analysis. Vitamin B-12 concentrations were measured via chemiluminescence; RBC and serum folate concentrations were evaluated via microbiological assay. Anemia and severe anemia were defined as hemoglobin <12.0 g/dL and <8.0 g/dL, respectively. Vitamin B-12 deficiency and insufficiency were defined as total vitamin B-12 <148 pmol/L and <221 pmol/L, respectively. Folate deficiency and insufficiency were defined as RBC folate <305 nmol/L and <748 nmol/L. A previously developed Bayesian model was used to predict neural tube defect (NTD) prevalence per 10,000 births.

Results: A total of 41.5% of WRA had anemia and 3.0% had severe anemia. A total of 48.3% of WRA had vitamin B-12 deficiency and 74.3% had vitamin B-12 insufficiency. The prevalence of RBC folate deficiency was 7.6%, and 79.3% of WRA had RBC folate <748 nmol/L, the threshold for optimal NTD prevention. Predicted NTD prevalence per 10,000 births based on RBC folate concentrations was 20.6 (95% uncertainty interval: 16.5–25.5).

Conclusions: The substantial burden of anemia, vitamin B-12 deficiency, and RBC folate insufficiency in WRA in this setting suggests an opportunity for anemia and birth defects prevention. Findings will directly inform the development of a randomized trial for anemia and birth defects prevention in southern India. This study was registered at clinicaltrials.gov as NCT04048330. *Curr Dev Nutr* 2021;5:nzab069.

Keywords: anemia, folate, vitamin B-12, NTDs, periconceptional, surveillance, India

Manuscript received February 27, 2021. Initial review completed April 12, 2021. Revision accepted April 20, 2021. Published online April 26, 2021.
This study was supported by the Centers for Disease Control and Prevention (CDC); the Division of Nutritional Sciences, Cornell University; and the University of South Carolina's Disability Research and Dissemination Center (DRDC) through its cooperative agreement (6U19DD001218) with the CDC. AF was supported by the National Institutes of Health under award 5 T32 HD087137.
Author disclosures: The authors report no conflicts of interest. The content is solely the responsibility of the authors and does not necessarily represent the official positions of the CDC, DRDC, Eunice Kennedy Shriver National Institute of Child Health and Human Development, or the National Institutes of Health.
Supplemental Tables 1–6 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/cdn/.

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Abbreviations used: ASF, animal-source food; GM, geometric mean; IEC, Institutional Ethics Committee; INR, Indian rupees; K2EDTA, di-potassium EDTA; LOD, limit(s) of detection; MBA, Microbiological Assay; NFHS, National Family Health Survey; NTD, neural tube defect; UI, uncertainty interval; WRA, women of reproductive age.

Introduction

Women of reproductive age (WRA) are a high-risk population for anemia and micronutrient deficiencies (1–3). Anemia is common in WRA and is associated with increased risk of maternal and infant mortality, low birth weight, and preterm birth (4–10). Deficiencies in vitamin B-12 and/or folate result in megaloblastic anemia (11, 12); inadequate folate status periconceptionally causes neural tube defects (NTDs) and there is emerging evidence that vitamin B-12 may be an independent risk factor for NTDs (13–15).

NTDs are structural birth defects associated with the failure of neural tube closure during embryonic development. There are >260,000 new NTD cases per year globally; this estimate varies from 1 to 80 NTDs per 10,000 births, with a higher prevalence in low and

middle-income countries (16–22). It is estimated that the burden of NTDs in India is among the highest globally (18, 23, 24); however, representative population-level data can establish the prevalence of NTDs and biomarkers that predict NTD risk.

The association between lower periconceptional folate status and increased risk of NTDs was first observed over 50 years ago (25, 26). Randomized trials demonstrated that periconceptional folic acid supplementation reduces the risk and recurrence of NTDs (15, 27–29). This led to dietary intake recommendations by the Institute of Medicine for WRA in the United States and folic acid fortification of flour in >80 countries (30). Fortification of staple foods with folic acid has been associated with a reduction in NTDs (31–34). In order to evaluate population-level NTD risk, the WHO recommends monitoring RBC folate concentrations in WRA (35, 36). This recommendation is supported by evidence from studies in the United States, China, and Ireland, which found that increases in RBC folate concentrations were associated with up to a 10-fold reduction in NTD risk (37–39).

Emerging evidence from laboratory and small observational studies has identified maternal vitamin B-12 deficiency as a risk factor for NTDs (13, 14) and other adverse pregnancy outcomes, including spontaneous abortion and low birth weight (13, 40, 41). Vitamin B-12 may also modify folate biomarkers that predict NTD risk at the population level (38). The burden of vitamin B-12 deficiency in India is estimated to be the highest in the world (41–44). The high prevalence of vitamin B-12 deficiency, and increasing evidence that low periconceptional vitamin B-12 status is a risk factor for NTDs, has stimulated interest in mandatory food fortification with vitamin B-12 (45). However, there are limited representative population-based data on the burden of vitamin B-12 and folate deficiencies in southern India (46). Surveillance programs can help establish critical biomarker data and inform interventions to improve maternal and child health.

The objective of this study was to conduct a biomarker survey of anemia and vitamin B-12 and folate status in WRA, as part of a periconceptional surveillance program in southern India. Findings from this biomarker survey will directly inform the development of a randomized trial of quadruple-fortified salt (i.e., iodine, iron, folic acid, vitamin B-12) for the prevention of anemia and birth defects in southern India.

Methods

Study design

This biomarker survey was conducted as part of a periconceptional surveillance program in southern India. The detailed design of this study has been previously described (47). Briefly, a census was conducted of all households within a 50-km^2 catchment area of our research site, Arogyavaram Medical Centre. Data collection was completed for all households within the catchment area (n = 6552 households; rural: 3124; urban: 3428).

Study population

Participants were eligible for the biomarker survey if they were female, aged 15–40 y, and were not pregnant or lactating. In order to obtain population-level biomarker data for WRA in this setting, all rural households (n = 3124) and a simple random sample of urban households (n = 1000) were selected for screening and recruitment. Trained nurse enumerators returned to selected households to confirm household member rosters and assess household member eligibility for the biomarker survey. For households with >1 eligible WRA, an a priori algorithm was used to randomly select 1 eligible WRA as the proband. Analyses included one WRA per household, but programmatically, all eligible WRA in the household were invited to participate in the biomarker survey. Research staff were blinded to the identity of the WRA proband until after data collection was completed. Women who were currently pregnant (self-reported, based on last menstrual period) or who had severe anemia (<8.0 g/dL) were referred to a local clinic for follow-up and standard of care.

Informed consent or assent

Written informed consent (≥18 y) or assent (15 to <18 y) was obtained from all participants prior to the start of data collection, with audio-visual recording in accordance with national regulations for clinical trials in India. If a woman was not able to read, a literate witness was asked to sign the form and the woman signed the form via a thumbprint.

Ethics

The study protocol was reviewed and approved by the Institutional Review Board at Cornell University, the Institutional Ethics Committee (IEC) at Arogyavaram Medical Centre, and the IEC at St John's Research Institute. The protocol was reviewed in accordance with CDC human research protection procedures and was determined to be a nonresearch, routine surveillance activity. A nondisclosure agreement for personally identifiable information and data-sharing agreement for de-identified data were established. This study received clearance from the Indian Council of Medical Research Health Ministry Screening Committee. The protocol was registered at ClincialTrials.gov (NCT04048330). Findings from this biomarker survey will directly inform the development of a randomized efficacy trial of quadruple-fortified salt (NCT03853304) in WRA in southern India. The protocol for the randomized trial was reviewed according to the CDC human research protection procedures and was determined to be research, but CDC involvement did not constitute engagement in human subjects research.

Data collection

Data were collected at Arogyavaram Medical Centre, the central research facility for the periconceptional surveillance program. Eligibility was confirmed (i.e., 15–40 y, not currently pregnant or lactating) before initiation of data collection. Transportation was provided for participants as needed. Individuals who missed their scheduled appointments were followed up to re-schedule appointments.

All data collection was conducted by trained nurse enumerators via interviewer-administered structured questionnaires on electronic tablets (48). Data collection procedures have been described in detail previously (47). Briefly, data collection (self-reported via interview) included sociodemographic data, dietary intake (24-h recall, including specific food items containing iron, vitamin B-12, and folate), anthropometric data, overall health, reproductive and pregnancy history (including birth defects and exposures that have been linked to increased risk of birth defects), and biological specimens (i.e., blood, saliva, urine). Anthropometric measurements, including

weight, height, midupper arm, waist, and hip circumferences, and triceps skinfold thickness, were measured in triplicate by trained research assistants.

Laboratory investigations

Biological specimen collection.

Blood samples were collected from each participant using standardized protocols. Venous blood (12 mL) was collected in three Vacutainers [i.e., red-top, purple-top di-potassium EDTA (K2EDTA), and blue-top metal-free K2EDTA; BD Vacutainers] for each participant. After collection, blood samples were immediately stored in a portable freezer unit that was set to optimal refrigeration temperature of 4 to 6°C, until processing within 4 h.

Sample processing and storage.

Red-top vacutainers were centrifuged (~1400 × *g*, 10 min, room temperature) to separate serum from cells, and serum was placed into aliquots and archived at −80°C or less until batch analysis. Purple-top Vacutainers were allowed to reach room temperature, re-mixed by inversion, and 100 μL of whole blood was added to 1 mL of 1% ascorbic acid to generate whole-blood lysate for the microbiological assay for assessment of RBC folate concentrations. Purple-top Vacutainers were analyzed for complete blood count, the remaining sample was centrifuged (~1400 × *g*, 10 min, room temperature), and plasma samples were stored at −80°C or less until batch analysis.

Laboratory analyses.

Laboratory analyses included hemoglobin, total vitamin B-12, and RBC and serum folate concentrations. Complete blood count (including hemoglobin) was analyzed using an automated Coulter counter (Coulter HMX). Serum total vitamin B-12 concentrations were assessed via chemiluminescence (E411; Roche Diagnostics). RBC folate and serum folate concentrations were measured using the WHO-recommended microbiological assay.

Definitions of outcomes

Anemia was defined as hemoglobin <12.0 g/dL, and severe anemia was defined as hemoglobin <8.0 g/dL (49). Vitamin B-12 deficiency and insufficiency were defined as total vitamin B-12 <148 pmol/L and <221 pmol/L, respectively (41, 50). Folate deficiency was defined as RBC folate <305 nmol/L, and folate insufficiency was defined as RBC folate <748 nmol/L, the recommended calibrator-adjusted equivalent of the threshold for optimal prevention of NTDs (36, 51).

Statistical analyses

Continuous variables were defined using conventional cutoffs, where available. Continuous biomarker and household income variables were natural logarithmically transformed for all analyses. Hemoglobin was adjusted for smoking status (self-reported). For biomarker analyses, values that were outside of the assay limits of detection (LOD) were set to half the LOD (if below the LOD) or 2 times the LOD (if above the LOD). Geometric means (GMs) and 95% CIs were calculated to facilitate statistical inference. Chi-square tests for contingency tables and 1-factor ANOVA were used to evaluate differences in categorial and continuous variables, respectively, and *P* values <0.05 were considered significant. All rural households and a random sample of urban households were included in unweighted totals. Population weights were constructed to account for differences in the study sample compared with the overall surveillance population and were used to calculate overall weighted population characteristics. Preliminary analyses indicated there were no substantial differences between the weighted and unweighted analyses and all results presented are for the unweighted analyses. We used the RBC folate distributions and vitamin B-12 status in this population and a previously developed Bayesian model (37) to predict NTD prevalence per 10,000 births (37). Statistical analyses were conducted using SAS version 9.4 (SAS Institute, Inc.).

Results

Characteristics of participants in this study are presented in **Table 1**, and a flowchart of households and participants is presented in **Figure 1**. The overall characteristics of the catchment area and households within the periconceptional surveillance program are described in **Supplemental Tables 1** and **2**. On average, there were 4.5 family members per household, and half of households reported a monthly income <5500 Indian rupees (INR; 1 US dollar ≅ 70 INR). Participating WRA (aged 15–40 y who were not pregnant or lactating) had a mean age of 28.8 y, and most women had some formal education (83.8%), although few reported finishing high school (10.9%) or college (15.7%). Women residing in rural or urban households were similar in terms of sociodemographic characteristics (e.g., age, household size, marital status, education, parity) (Table 1).

Anthropometric and dietary characteristics of participants are presented in **Table 2**. Among adult women (≥18 y), 19.2% were underweight [BMI (kg/m^2) <18.5], 23.4% were overweight (BMI: 25.0 to <30.0), and 9.6% had obesity (BMI ≥30.0). Women in urban areas had significantly higher BMI ($P = 0.01$), midupper arm circumference ($P = 0.03$), and waist circumference ($P = 0.03$). The prevalence of veganism (<1%) or vegetarianism (6.6%) was low, with 93.1% of WRA self-reporting as nonvegetarian; however, overall, the frequency of consumption of animal-source foods (ASFs; e.g., egg, poultry, meat, and fish) was low in both vegetarian and nonvegetarian individuals. WRA residing in urban areas were significantly more likely to be nonvegetarian (urban vs. rural: 99.5% vs. 91.6%; $P = 0.0001$) and reported more frequent consumption of ASFs compared with women in rural areas (Table 2).

The prevalences of anemia, vitamin B-12, and folate status in WRA are presented in **Table 3**. A total of 41.5% of women had anemia (<12.0 g/dL) and 3.0% had severe anemia (<8.0 g/dL), with mean hemoglobin concentrations of 11.9 g/dL (95% CI: 11.7, 12.0 g/dL). The prevalence of vitamin B-12 deficiency (<148 pmol/L) was 48.3%, and 74.3% of WRA were vitamin B-12 insufficient (<221 pmol/L); mean vitamin B-12 concentration was 156.0 pmol/L (95% CI: 150.3, 162.0 pmol/L). The mean serum folate concentration in this population was 16.5 nmol/L (95% CI: 16.0, 17.0 nmol/L); 3.5% of women were serum folate deficient (<7.0 nmol/L). Mean RBC folate concentrations were 540.5 nmol/L (95% CI: 526.2, 555.1 nmol/L). The prevalence of RBC folate deficiency (<305 nmol/L) was 7.6%, and 79.3% of WRA had RBC folate concentrations <748 nmol/L, the threshold for optimal NTD prevention.

TABLE 1 Sociodemographic characteristics of the study population[1]

Variables	*n*	GM (95% CI) or *n* (%)			*P*[2]
		Total (*n* = 980)	Rural (*n* = 788)	Urban (*n* = 192)	
Household					
Household size	980	4.5 (4.4, 4.7)	4.6 (4.4, 4.7)	4.4 (4.1, 4.7)	0.25
Monthly household income,[3] INR	979	5452.1 (5260.9, 5650.2)	5474.3 (5260.7, 5696.6)	5361.8 (4946.6, 5811.8)	0.65
Type of house	980				
Kutcha		41 (4.2)	27 (3.4)	14 (7.3)	0.04
Semi-pucca		3 (0.3)	3 (0.4)	0 (0.0)	
Pucca		936 (95.5)	758 (96.2)	178 (92.7)	
Purchases iodized salt	974	654 (67.1)	525 (67.0)	129 (67.5)	0.66
Woman of reproductive age					
Age, y	980	28.8 (28.4, 29.3)	28.8 (28.3, 29.3)	29.1 (28.1, 30.2)	0.57
15 to <18 y		49 (5.0)	41 (5.2)	8 (4.2)	0.81
18 to <26 y		229 (23.4)	187 (23.7)	42 (21.9)	
26 to <36 y		453 (46.2)	359 (45.6)	94 (49.0)	
36 to 40 y		249 (25.4)	201 (25.5)	48 (25.0)	
Highest level of education	975				
No formal schooling		158 (16.2)	125 (15.9)	33 (17.3)	0.65
Grades 1–5		174 (17.8)	138 (17.6)	36 (18.8)	
Grades 6–8		177 (18.2)	142 (18.1)	35 (18.3)	
Grades 9–10		207 (21.2)	165 (21.0)	42 (22.0)	
Grades 11–12		106 (10.9)	83 (10.6)	23 (12.0)	
College or graduate degree		153 (15.7)	131 (16.7)	22 (11.5)	
Marital status	975				
Currently married		774 (79.4)	617 (78.7)	157 (82.2)	0.56
Widowed, divorced, separated		35 (3.6)	29 (3.7)	6 (3.1)	
Never married		166 (17.0)	138 (17.6)	28 (14.7)	
Gravidity	975	2.0 (1.9, 2.1)	2.0 (1.9, 2.1)	2.0 (1.8, 2.3)	0.72
0		207 (21.2)	169 (21.6)	38 (19.9)	0.84
1		68 (7.0)	53 (6.8)	15 (7.9)	
2		385 (39.5)	306 (39.0)	79 (41.4)	
≥3		315 (32.3)	256 (32.7)	59 (30.9)	
Parity	975	1.7 (1.6, 1.8)	1.7 (1.6, 1.8)	1.6 (1.5, 1.8)	0.68
Nulliparous		231 (23.7)	187 (23.9)	44 (23.0)	0.41
Primiparous		88 (9.0)	66 (8.4)	22 (11.5)	
Multiparous		656 (67.3)	531 (67.7)	125 (65.4)	
Currently has children	746	737 (98.8)	592 (99.0)	145 (98.0)	0.31
Number of children		2.0 (1.9, 2.1)	2.0 (1.9, 2.1)	1.9 (1.7, 2.2)	0.54
0		9 (1.2)	6 (1.0)	3 (2.0)	0.43
1		118 (15.8)	90 (15.1)	28 (18.9)	
2		514 (68.9)	415 (69.4)	99 (66.9)	
≥3		105 (14.1)	87 (14.5)	18 (12.2)	

[1]Values are GMs (95% CI) or *n* (%). Total (*n*s) are true sample *n*s. GM, geometric mean; INR, Indian rupees; USD, US dollar.
[2]Chi-square statistics and 1-factor ANOVA were used to evaluate differences in categorical and continuous variables, respectively; household income was natural logarithmically transformed prior to analyses; Poisson regressions were used for count variables (e.g., household size, gravidity, parity, and number of children)
[3]INR 70 ≅ 1 USD.

The prevalence of concurrent vitamin B-12 and folate deficiency and combinations of vitamin B-12 and folate status in WRA are summarized in Table 3. A total of 10.0% of women had adequate vitamin B-12 and RBC folate status; in contrast, 51.1% of women were vitamin B-12 or RBC folate deficient, and 90.1% of participants had either vitamin B-12 or RBC folate insufficiency. A total of 5.3% or 10.8% of women had sufficient RBC folate (≥748 nmol/L) status in combination with vitamin B-12 deficiency or vitamin B-12 insufficiency, respectively.

Anemia and vitamin B-12 and folate statuses in WRA, stratified by rural or urban residence, are also presented in Table 3. Hemoglobin and vitamin B-12 concentrations were not significantly different between rural and urban strata. RBC folate concentrations ($P = 0.0009$) were significantly lower among women residing in rural areas compared with those in urban areas [rural vs. urban: GM 528.5 nmol/L (95% CI: 513.0, 544.4) vs. 592.3 nmol/L (95% CI: 557.8, 628.9)]. In contrast, serum folate concentrations were higher in women residing in rural compared with urban settings ($P = 0.0007$). The prevalence of anemia (rural vs. urban: 42.2% vs. 38.5%), vitamin B-12 deficiency (rural vs. urban: 49.1% vs. 44.8%), vitamin B-12 insufficiency (rural vs. urban: 74.2% vs. 75.0%), and RBC folate deficiency (rural vs. urban: 7.9% vs. 6.3%) or serum folate deficiency (rural vs. urban: 3.6% vs. 3.1%) were not significantly different comparing women residing in rural and urban areas ($P > 0.05$). However, the prevalence of RBC folate insufficiency (<748 nmol/L) was significantly higher in women residing in rural areas compared with those in urban areas (rural vs. urban: 80.9% vs. 72.9%; $P = 0.01$); WRA residing in rural areas had a 1.11 times greater risk of RBC folate

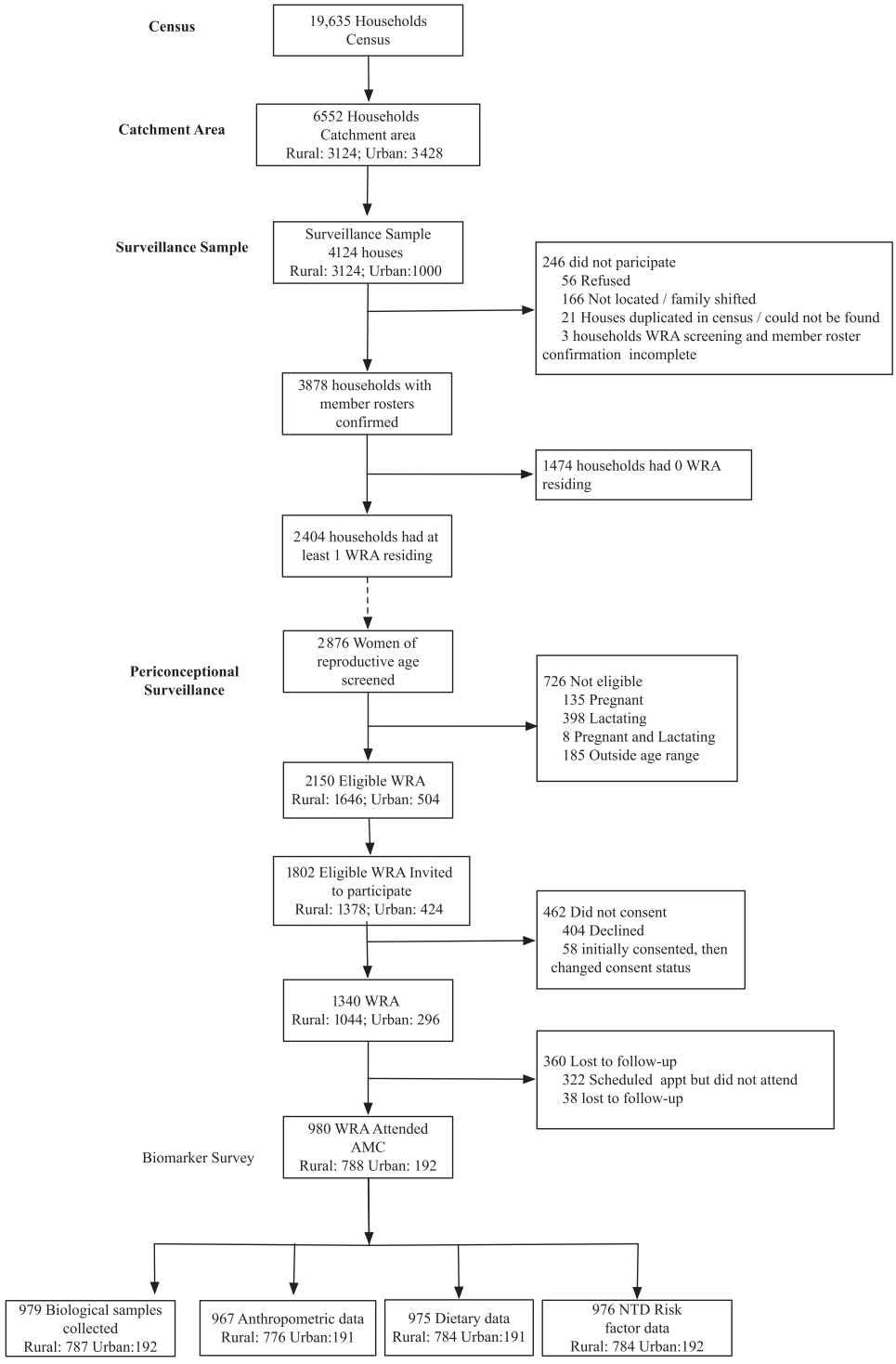

FIGURE 1 Participant flow chart. AMC, Arogyavaram Medical Center; appt, appointment; NTD, neural tube defect; WRA, women of reproductive age.

insufficiency (RR: 1.11; 95% CI: 1.01, 1.22; $P = 0.03$; data not shown) compared with those in urban areas.

Sociodemographic characteristics by vitamin B-12 status in WRA are presented in **Table 4**. Household size, household income, type of house, purchasing of iodized salt, WRA level of education, and WRA gravidity, parity, and number of children were similar across vitamin B-12–deficient and not vitamin B-12–deficient strata. Women who had vitamin B-12 deficiency were younger ($P = 0.04$) and less likely to

TABLE 2 Anthropometric and dietary characteristics of the study population[1]

Variables	*n*	GM (95% CI) or *n* (%)			*P*[2]
		Total (*n* = 980)	Rural (*n* = 788)	Urban (*n* = 192)	
Anthropometric					
Weight, kg	969	53.0 (52.2, 53.7)	52.6 (51.7, 53.4)	54.6 (52.8, 56.4)	0.06
Height, cm	969	153.2 (152.9, 153.6)	153.4 (153.0, 153.8)	152.7 (151.9, 153.5)	0.14
BMI, kg/m^2	969	22.6 (22.2, 22.9)	22.3 (22.0, 22.7)	23.4 (22.7, 24.2)	0.01
BMI (kg/m^2)[3,4]	920				
<18.5		177 (19.2)	153 (20.8)	24 (13.1)	0.02
18.5 to <25.0		440 (47.8)	354 (48.0)	86 (47.0)	
25.0 to <30.0		215 (23.4)	159 (21.6)	56 (30.6)	
≥30.0		88 (9.6)	71 (9.6)	17 (9.3)	
BMI (kg/m^2)[3,5]	920				
<18.5		177 (19.2)	153 (20.8)	24 (13.1)	0.04
18.5 to <23.0		308 (33.5)	250 (33.9)	58 (31.7)	
23.0 to <27.5		253 (27.5)	198 (26.9)	55 (30.1)	
≥27.5		182 (19.8)	136 (18.5)	46 (25.1)	
Midupper arm circumference, cm	969	26.7 (26.5, 27.0)	26.6 (26.3, 26.9)	27.3 (26.7, 27.9)	0.03
Waist circumference, cm	969	74.6 (73.9, 75.3)	74.2 (73.4, 75.0)	76.3 (74.7, 78.0)	0.03
>88.9 cm[3,6]	920	123 (13.4)	93 (12.6)	30 (16.4)	0.18
Dietary preference[7]	974				
Vegan		3 (0.3)	3 (0.4)	0 (0.0)	0.001
Vegetarian		64 (6.6)	63 (8.0)	1 (0.5)	
Nonvegetarian		907 (93.1)	717 (91.6)	190 (99.5)	
Dietary preference[7]	974				
Vegan or vegetarian		67 (6.9)	66 (8.4)	1 (0.5)	0.0001
Nonvegetarian		907 (93.1)	717 (91.6)	190 (99.5)	
Frequency of animal-source foods consumption					
Dairy	974				
Never		53 (5.4)	42 (5.4)	11 (5.8)	0.02
Almost never (<1×/mo)		35 (3.6)	35 (4.5)	0 (0.0)	
Occasionally (<1×/wk)		79 (8.1)	63 (8.0)	16 (8.4)	
Often (~1×/wk)		152 (15.6)	114 (14.6)	38 (19.9)	
Very often (>1×/wk)		655 (67.2)	529 (67.6)	126 (66.0)	
Eggs	969				
Never		72 (7.4)	65 (8.4)	7 (3.7)	0.003
Almost never (<1×/mo)		107 (11.0)	95 (12.2)	12 (6.3)	
Occasionally (<1×/wk)		178 (18.4)	134 (17.2)	44 (23.0)	
Often (~1×/wk)		376 (38.8)	289 (37.1)	87 (45.5)	
Very often (>1×/wk)		236 (24.4)	195 (25.1)	41 (21.5)	
Poultry	973				
Never		106 (10.9)	99 (12.7)	7 (3.7)	0.0007
Almost never (<1×/mo)		81 (8.3)	67 (8.6)	14 (7.3)	
Occasionally (<1×/wk)		119 (12.2)	100 (12.8)	19 (9.9)	
Often (~1×/wk)		537 (55.2)	409 (52.3)	128 (67.0)	
Very often (>1×/wk)		130 (13.4)	107 (13.7)	23 (12.0)	
Meat	973				
Never		219 (22.5)	197 (25.2)	22 (11.5)	<0.0001
Almost never (<1×/mo)		303 (31.1)	240 (30.7)	63 (33.0)	
Occasionally (<1×/wk)		213 (21.9)	148 (18.9)	65 (34.0)	
Often (~1×/wk)		209 (21.5)	169 (21.6)	40 (20.9)	
Very often (>1×/wk)		29 (3.0)	28 (3.6)	1 (0.5)	
Fish	973				
Never		308 (31.7)	261 (33.4)	47 (24.6)	0.04
Almost never (<1×/mo)		388 (39.9)	298 (38.1)	90 (47.1)	
Occasionally (<1×/wk)		178 (18.3)	139 (17.8)	39 (20.4)	
Often (~1×/wk)		74 (7.6)	61 (7.8)	13 (6.8)	
Very often (>1×/wk)		25 (2.6)	23 (2.9)	2 (1.0)	

[1]Values GMs (95% CI) or *n* (%). Total (*ns*) are true sample *n*s. GM, geometric mean.
[2]Chi-square statistics and 1-factor ANOVA were used to evaluate differences in categorical and continuous variables, respectively.
[3]Among participants ≥18 y old (*n* = 931; rural: 747; urban: 184).
[4]BMI categories as defined by the WHO (52).
[5]BMI categories for Asian populations (53).
[6]Defined as >35 inches, converted to >88.9 cm (54).
[7]Vegetarian: consumed milk and/or eggs; Nonvegetarian: consumed poultry, meat, and/or fish.

TABLE 3 Anemia and vitamin B-12 and folate status in women of reproductive age[1]

		GM (95% CI) or *n* (%)			
	n	**Total (*n* = 979)**	**Rural (*n* = 787)**	**Urban (*n* = 192)**	***P*[2]**
Hemoglobin,[3] g/dL	979	11.9 (11.7, 12.0)	11.8 (11.7, 11.9)	12.1 (11.8, 12.4)	0.07
<12.0 g/dL		406 (41.5)	332 (42.2)	74 (38.5)	0.36
<8.0 g/dL		29 (3.0)	27 (3.4)	2 (1.0)	0.08
RBC folate,[4] nmol/L	977	540.5 (526.2, 555.1)	528.5 (513.0, 544.4)	592.3 (557.8, 628.9)	0.0009
<305 nmol/L		74 (7.6)	62 (7.9)	12 (6.3)	0.44
<748 nmol/L		775 (79.3)	635 (80.9)	140 (72.9)	0.01
Serum folate,[4] nmol/L	977	16.5 (16.0, 17.0)	17.0 (16.4, 17.6)	14.8 (13.8, 15.9)	0.0007
<7 nmol/L		34 (3.5)	28 (3.6)	6 (3.1)	0.76
Serum vitamin B-12, pmol/L	978	156.0 (150.3, 162.0)	155.0 (148.7, 161.6)	160.2 (147.3, 174.4)	0.49
<148 pmol/L		472 (48.3)	386 (49.1)	86 (44.8)	0.28
<221 pmol/L		727 (74.3)	583 (74.2)	144 (75.0)	0.81
Vitamin B-12 and RBC folate deficient		47 (4.8)	39 (5.0)	8 (4.2)	0.64
Vitamin B-12 and RBC folate insufficient		622 (63.7)	510 (65.0)	112 (58.3)	0.09
Vitamin B-12 or RBC folate deficient		499 (51.1)	409 (52.1)	90 (46.9)	0.19
Vitamin B-12 or RBC folate insufficient		880 (90.1)	708 (90.2)	172 (89.6)	0.80
Vitamin B-12 ≥148 pmol/L and RBC folate <748 nmol/L		355 (36.3)	285 (36.3)	70 (36.5)	0.97
Vitamin B-12 ≥221 pmol/L and RBC folate <748 nmol/L		153 (15.7)	125 (15.9)	28 (14.6)	0.65
Vitamin B-12 <148 pmol/L and RBC folate ≥748 nmol/L		52 (5.3)	36 (4.6)	16 (8.3)	0.04
Vitamin B-12 <221 pmol/L and RBC folate ≥748 nmol/L		105 (10.8)	73 (9.3)	32 (16.7)	0.003

[1]Values are GMs (95% CI) or *n* (%). Total (*ns*) are true sample *ns*. Results outside assay limits of detection (LOD) were set to 0.50 × LOD (if below LOD) or 2 × LOD (if above LOD); results outside assay LODs: serum folate (*n* = 1 below LOD), vitamin B-12 (*n* = 4 below LOD, *n* = 6 above LOD). GM, geometric mean.
[2]Chi-square statistics and 1-factor ANOVA were used to evaluate differences in categorical and continuous variables, respectively; hemoglobin, serum folate, RBC folate, and vitamin B-12 were natural logarithmically transformed prior to analyses.
[3]Hemoglobin values from complete blood count; adjusted for smoking status (49).
[4]Microbiological assay.

TABLE 4 Sociodemographic characteristics of the study population by vitamin B-12 deficiency (<148 pmol/L)[1]

Variables	*n*	GM (95% CI) or *n* (%)		*P*[2]
		Vitamin B-12 deficient (*n* = 472)	Not vitamin B-12 deficient (*n* = 506)	
Household				
Household size	978	4.5 (4.3, 4.7)	4.6 (4.4, 4.7)	0.79
Rural location	978	386 (81.8)	400 (79.1)	0.28
Monthly household income,[3] INR	977	5490.6 (5215.0, 5780.8)	5421.3 (5158.5, 5697.5)	0.73
Type of house	978			
Kutcha		21 (4.4)	20 (4.0)	0.18
Semi-pucca		3 (0.6)	0 (0.0)	
Pucca		448 (94.9)	486 (96.0)	
Household purchases iodized salt	972	327 (69.4)	327 (65.3)	0.30
Woman of reproductive age				
Age, y	978	28.3 (27.6, 28.9)	29.4 (28.7, 30.0)	0.04
15 to <18 y		33 (7.0)	16 (3.2)	0.04
18 to <26 y		110 (23.3)	118 (23.3)	
26 to <36 y		218 (46.2)	235 (46.4)	
36 to 40 y		111 (23.5)	137 (27.1)	
Highest level of education completed	973			
No formal schooling		69 (14.6)	89 (17.7)	0.42
Grades 1–5		80 (17.0)	93 (18.5)	
Grades 6–8		84 (17.8)	93 (18.5)	
Grades 9–10		100 (21.2)	107 (21.3)	
Grades 11–12		54 (11.5)	52 (10.4)	
College or graduate degree		84 (17.8)	68 (13.5)	
Marital status	973			
Currently married		357 (75.8)	416 (82.9)	0.002
Widowed, divorced, separated		14 (3.0)	21 (4.2)	
Never married		100 (21.2)	65 (12.9)	
Gravidity	973	1.9 (1.8, 2.1)	2.1 (2.0, 2.2)	0.06
0		113 (24.0)	93 (18.5)	0.17
1		29 (6.2)	38 (7.6)	
2		185 (39.3)	200 (39.8)	
≥3		144 (30.6)	171 (34.1)	
Parity	973	1.6 (1.5, 1.7)	1.7 (1.6, 1.9)	0.10
Nulliparous		123 (26.1)	106 (21.1)	0.18
Primiparous		42 (8.9)	46 (9.2)	
Multiparous		306 (65.0)	350 (69.7)	
Currently has children	746	347 (99.1)	390 (98.5)	0.41
Number of children		2.0 (1.8, 2.1)	2.0 (1.9, 2.1)	0.68
0		3 (0.9)	6 (1.5)	0.54
1		61 (17.4)	57 (14.4)	
2		240 (68.6)	274 (69.2)	
≥3		46 (13.1)	59 (14.9)	

[1]Vitamin B-12 missing for *n* = 1 woman of reproductive age. Results outside assay limits of detection (LOD) were set to 0.50 × LOD (if below LOD) or 2 × LOD (if above LOD); results outside assay LODs: vitamin B-12 (*n* = 4 below LOD, *n* = 6 above LOD). GM, geometric mean; INR, Indian rupees; USD, US dollar.
[2]Chi-square statistics and 1-factor ANOVA were used to evaluate differences in categorical and continuous variables, respectively; household income was natural logarithmically transformed prior to analyses; Poisson regressions were used for count variables (e.g., household size, gravidity, parity, and number of children).
[3]INR 70 ≅ 1 USD.

report being married (vitamin B-12 deficient vs. not vitamin B-12 deficient: 75.8% vs. 82.9%; $P = 0.002$). Dietary patterns by vitamin B-12 status in WRA are presented in **Table 5**. Women with vitamin B-12 deficiency were significantly more likely to be vegetarian/vegan compared with women who were not vitamin B-12 deficient (vitamin B-12 deficient vs. not vitamin B-12 deficient: 9.8% vs. 4.2%; $P = 0.0006$). In terms of ASF intake, WRA who were vitamin B-12 deficient reported similar frequency of dairy, egg, and fish consumption compared with WRA who were not vitamin B-12 deficient ($P > 0.05$) but had significantly lower frequency of poultry ($P = 0.006$) and meat ($P = 0.001$) intakes. WRA who were vegetarian/vegan had 1.47 times greater risk of vitamin B-12 deficiency compared with women who were nonvegetarian (RR: 1.47; 95% CI: 1.23,1.75; $P < 0.0001$; data not shown).

Sociodemographic characteristics by RBC folate status in WRA are presented in **Table 6**. Household size, household income, and purchasing of iodized salt were similar across RBC folate–insufficient and RBC folate–sufficient strata. Women who were RBC folate insufficient were younger ($P = 0.0001$), more likely to live in rural households (RBC folate insufficient vs. RBC folate sufficient: 81.9% vs. 74.3%; $P = 0.01$), less likely to report being married (RBC folate insufficient vs. RBC folate sufficient: 77.4% vs. 87.1%; $P = 0.003$), and reported fewer pregnancies ($P = 0.02$) compared with women with RBC folate sufficiency. There

TABLE 5 Dietary characteristics of the study population by vitamin B-12 deficiency (<148 pmol/L)[1]

Variables	*n*	GM (95% CI) or *n* (%)		*P*[2]
		Vitamin B-12 deficient (*n* = 472)	Not vitamin B-12 deficient (*n* = 506)	
Dietary preference[3]	972			
Vegan		2 (0.4)	1 (0.2)	0.003
Vegetarian		44 (9.4)	20 (4.0)	
Nonvegetarian		424 (90.2)	481 (95.8)	
Dietary preference[3]	972			
Vegan or vegetarian		46 (9.8)	21 (4.2)	0.0006
Nonvegetarian		424 (90.2)	481 (95.8)	
Frequency of animal-source foods consumption				
Dairy	972			
Never		23 (4.9)	30 (6.0)	0.55
Almost never (<1×/mo)		14 (3.0)	21 (4.2)	
Occasionally (<1×/wk)		40 (8.5)	38 (7.6)	
Often (~1×/wk)		68 (14.5)	84 (16.7)	
Very often (>1×/wk)		325 (69.1)	329 (65.5)	
Eggs	967			
Never		46 (9.8)	26 (5.2)	0.07
Almost never (<1×/mo)		49 (10.5)	58 (11.6)	
Occasionally (<1×/wk)		82 (17.5)	96 (19.2)	
Often (~1×/wk)		184 (39.3)	190 (38.1)	
Very often (>1×/wk)		107 (22.9)	129 (25.9)	
Poultry	971			
Never		67 (14.3)	38 (7.6)	0.006
Almost never (<1×/mo)		36 (7.7)	45 (9.0)	
Occasionally (<1×/wk)		55 (11.7)	64 (12.8)	
Often (~1×/wk)		260 (55.3)	276 (55.1)	
Very often (>1×/wk)		52 (11.1)	78 (15.6)	
Meat	971			
Never		128 (27.3)	91 (18.1)	0.001
Almost never (<1×/mo)		153 (32.6)	150 (29.9)	
Occasionally (<1×/wk)		91 (19.4)	121 (24.1)	
Often (~1×/wk)		88 (18.8)	120 (23.9)	
Very often (>1×/wk)		9 (1.9)	20 (4.0)	
Fish	971			
Never		162 (34.5)	146 (29.1)	0.18
Almost never (<1×/mo)		185 (39.4)	202 (40.3)	
Occasionally (<1×/wk)		84 (17.9)	93 (18.6)	
Often (~1×/wk)		31(6.6)	43 (8.6)	
Very often (>1×/wk)		8 (1.7)	17 (3.4)	

[1]Vitamin B-12 missing for *n* = 1 woman of reproductive age. Results outside assay limits of detection (LOD) were set to 0.50 × LOD (if below LOD) or 2 × LOD (if above LOD); results outside assay LODs: vitamin B-12 (*n* = 4 below LOD, *n* = 6 above LOD). GM, geometric mean.
[2]Chi-square statistics were used to evaluate differences in categorical variables.
[3]Vegetarian: consumed milk and/or eggs; Nonvegetarian: consumed poultry, meat, and/or fish.

were no differences in RBC folate insufficiency among self-reported dietary patterns (vegetarian vs. nonvegetarian) (Table 7).

Sociodemographic characteristics by anemia and serum folate status in WRA are presented in the supplemental tables (**Supplemental Tables 3–6**). There were no differences in anemia among self-reported dietary patterns (vegetarian vs. nonvegetarian) or reported consumption of ASFs (i.e., egg, dairy, poultry, or meat products; $P > 0.05$), except for fish products ($P = 0.03$); all other sociodemographic characteristics were similar by anemia strata (Supplemental Tables 3 and 4) and by serum folate deficiency strata (Supplemental Tables 5 and 6).

Overall, the predicted NTD prevalence per 10,000 births based on RBC folate concentrations was 20.6 [95% uncertainty interval (UI): 16.5–25.5]. Predicted NTD prevalence in rural WRA was 21.3 per 10,000 births (95% UI: 16.9–26.3) and was 18.2 per 10,000 births (95% UI: 14.4–22.6) in urban WRA. The predicted NTD prevalences in rural and urban strata were not significantly different: the median difference between these 2 groups was 3.1 NTDs per 10,000 births, with a 0.95 probability of the true value being between −1.0 and 7.0.

Discussion

In this population-based biomarker survey among WRA, there was a high burden of anemia, vitamin B-12 deficiency, and RBC folate insufficiency. A total of 41.5% of WRA had anemia; 48.3% had vitamin B-12 deficiency, and 74.3% had vitamin B-12 insufficiency. Although the prevalence of RBC folate deficiency was 7.6%, 79.3% of women had RBC folate concentrations below the threshold for optimal NTD prevention. The substantial burden of anemia, vitamin B-12 deficiency, and RBC folate insufficiency in this population suggests an opportunity for anemia

TABLE 6 Sociodemographic characteristics of the study population by RBC folate insufficiency (<748 nmol/L)[1]

Variables	*n*	GM (95% CI) or *n* (%)		*P*[2]
		RBC folate insufficient (*n* = 775)	RBC folate sufficient (*n* = 202)	
Household				
Household size	977	4.6 (4.4, 4.7)	4.5 (4.2, 4.8)	0.53
Rural location	977	635 (81.9)	150 (74.3)	0.01
Monthly household income,[3] INR	976	5393.1 (5180.7, 5614.1)	5702.4 (5271.3, 6168.9)	0.22
Type of house	977			
Kutcha		35 (4.5)	6 (3.0)	0.42
Semi-pucca		3 (0.4)	0 (0.0)	
Pucca		737 (95.1)	196 (97.0)	
Household purchases iodized salt	971	521 (67.7)	132 (65.7)	0.33
Woman of reproductive age				
Age, y	977	28.4 (27.9, 28.9)	30.6 (29.6, 31.7)	0.0001
15 to <18 y		45 (5.8)	4 (2.0)	0.004
18 to <26 y		192 (24.8)	36 (17.8)	
26 to <36 y		356 (45.9)	96 (47.5)	
36 to 40 y		182 (23.5)	66 (32.7)	
Highest level of education completed	972			
No formal schooling		129 (16.7)	29 (14.4)	0.03
Grades 1–5		139 (18.0)	34 (16.9)	
Grades 6–8		127 (16.5)	49 (24.4)	
Grades 9–10		157 (20.4)	50 (24.9)	
Grades 11–12		89 (11.5)	17 (8.5)	
College or graduate degree		130 (16.9)	22 (10.9)	
Marital status	972			
Currently married		597 (77.4)	175 (87.1)	0.003
Widowed, divorced, separated		27 (3.5)	8 (4.0)	
Never married		147 (19.1)	18 (9.0)	
Gravidity	972	2.0 (1.9, 2.1)	2.2 (2.0, 2.4)	0.02
0		178 (23.1)	28 (13.9)	0.04
1		51 (6.6)	16 (8.0)	
2		300 (38.9)	85 (42.3)	
≥3		242 (31.4)	72 (35.8)	
Parity	972	1.6 (1.5, 1.7)	1.8 (1.7, 2.0)	0.03
Nulliparous		194 (25.2)	35 (17.4)	0.06
Primiparous		70 (9.1)	18 (9.0)	
Multiparous		507 (65.8)	148 (73.6)	
Currently has children	745	571 (98.6)	165 (99.4)	0.42
Number of children		1.9 (1.9, 2.1)	2.0 (1.8, 2.3)	0.50
0		8 (1.4)	1 (0.6)	0.36
1		97 (16.8)	21 (12.7)	
2		397 (68.6)	116 (69.9)	
≥3		77 (13.3)	28 (16.9)	

[1]RBC folate missing for *n* = 2 women of reproductive age. Results outside assay limits of detection (LOD) were set to 0.50 × LOD (if below LOD) or 2 × LOD (if above LOD); results outside assay LODs: serum folate (*n* = 1 below LOD). GM, geometric mean; INR, Indian rupees; USD, US dollar.
[2]Chi-square statistics and 1-factor ANOVA were used to evaluate differences in categorical and continuous variables, respectively; household income was natural logarithmically transformed prior to analyses; Poisson regressions were used for count variables (e.g., household size, gravidity, parity, and number of children).
[3]INR 70 ≅ 1 USD.

and birth defects prevention through interventions such as the fortification of staple foods.

Anemia, vitamin B-12 deficiency, and folate insufficiencies have documented risks to WRA, their pregnancies, and children. The WHO estimates that ~33% of nonpregnant WRA are anemic worldwide (2016 estimates; 55), with the highest prevalence of 45.6% in the WHO–South-East Asia Regional Office region (55), including India [52.3%; National Family Health Survey (NFHS)-4, 2014–2015] (56). The prevalence of anemia (41.5%) and severe anemia (3.0%) in the current study is consistent with findings from studies in other parts of India where the reported prevalence of anemia in WRA ranged from 28% to 64% (57–60) and the prevalence of severe anemia ranged from 2.9% to 4.0% (58–60). The prevalence of anemia in the current study (41.5%) was slightly lower than the most recent NFHS-5 (NFHS-5, 2019–2020; nonpregnant WRA aged 15–49 y) district-level (Chittoor: 51.8%, NFHS-5, 2019–2020; 48.4%, NFHS-4, 2014–2015) and state-level (Andhra Pradesh: 59.0%; urban: 57.8%, rural: 59.5%; NHFS-4: 60.2%) data, although the use of capillary blood, inclusion of lactating women, and different time periods constrain direct comparability of findings (61, 62).

Vitamin B-12 deficiency in its most severe form is a cause of megaloblastic anemia and can be associated with fatigue and neurological manifestations (44). The high prevalence of vitamin B-12 deficiency

TABLE 7 Dietary characteristics of the study population by RBC folate insufficiency (<748 nmol/L)[1]

Variables	*n*	GM (95% CI) or *n* (%)		*P*[2]
		RBC folate insufficient (*n* = 775)	RBC folate sufficient (*n* = 202)	
Dietary preference[3]	971			
Vegan		2 (0.3)	1 (0.5)	0.86
Vegetarian		51 (6.6)	13 (6.5)	
Nonvegetarian		717 (93.1)	187 (93.0)	
Dietary preference[3]	971			
Vegan or vegetarian		53 (6.9)	14 (7.0)	0.97
Nonvegetarian		717 (93.1)	187 (93.0)	
Animal-source foods consumption				
Dairy	971			
Never		42 (5.5)	11 (5.5)	0.91
Almost never (<1×/mo)		30 (3.9)	5 (2.5)	
Occasionally (<1×/wk)		61 (7.9)	17 (8.5)	
Often (~1×/wk)		121 (15.7)	31 (15.4)	
Very often (>1×/wk)		516 (67.0)	137 (68.2)	
Eggs	966			
Never		57 (7.5)	15 (7.5)	0.75
Almost never (<1×/mo)		81 (10.6)	25 (12.4)	
Occasionally (<1×/wk)		142 (18.6)	36 (17.9)	
Often (~1×/wk)		303 (39.6)	71 (35.3)	
Very often (>1×/wk)		182 (23.8)	54 (26.9)	
Poultry	970			
Never		81 (10.5)	24 (11.9)	0.96
Almost never (<1×/mo)		64 (8.3)	17 (8.5)	
Occasionally (<1×/wk)		93 (12.1)	26 (12.9)	
Often (~1×/wk)		426 (55.4)	109 (54.2)	
Very often (>1×/wk)		105 (13.7)	25 (12.4)	
Meat	970			
Never		178 (23.1)	41 (20.4)	0.12
Almost never (<1×/mo)		250 (32.5)	52 (25.9)	
Occasionally (<1×/wk)		156 (20.3)	56 (27.9)	
Often (~1×/wk)		163 (21.2)	45 (22.4)	
Very often (>1×/wk)		22 (2.9)	7 (3.5)	
Fish	970			
Never		240 (31.2)	67 (33.3)	0.29
Almost never (<1×/mo)		314 (40.8)	73 (36.3)	
Occasionally (<1×/wk)		137 (17.8)	40 (19.9)	
Often (~1×/wk)		55 (7.2)	19 (9.5)	
Very often (>1×/wk)		23 (3.0)	2 (1.0)	

[1]RBC folate missing for *n* = 2 women of reproductive age. Results outside assay limits of detection (LOD) were set to 0.50 × LOD (if below LOD) or 2 × LOD (if above LOD); results outside assay LODs: serum folate (*n* = 1 below LOD). GM, geometric mean.
[2]Vegetarian: consumed milk and/or eggs; Nonvegetarian: consumed poultry, meat, and/or fish.
[3]Chi-square statistics were used to evaluate differences in categorical variables.

(<148 pmol/L; 48.3%) and insufficiency (<221 pmol/L; 74.3%) among WRA in this study is consistent with other studies among WRA in India (31–58%) (57, 63–65): in rural Nagpur (31%) (63), rural Telangana (45.0%) (57), and vegetarian WRA in Pune (50%) (65). There are limited population-based data on vitamin B-12 status worldwide, including in WRA (41, 44, 66–68). To date, 14 countries have conducted national surveys evaluating vitamin B-12 concentrations, with reported prevalences of vitamin B-12 deficiency in WRA ranging from 3.3% to 52.4% (66, 67, 69–82). The prevalence of vitamin B-12 deficiency in the current study is higher than in WRA in surveys conducted in most other settings (including the United States, Canada, Mexico, Argentina, Colombia, Ecuador, Guatemala, Costa Rica, Belize, the United Kingdom, Germany, Jordan, Bangladesh) (66, 67, 69–82), with prevalences ranging from 3.3% in the United States (69) to 24.1% in Mexico (71), although lower than national data in Pakistan (52.4%) (74).

In this study, the prevalence of vitamin B-12 deficiency was significantly higher in vegetarian/vegan WRA compared with nonvegetarian women ($P = 0.0006$): women who were vegetarian or vegan had 1.47 times greater risk of vitamin B-12 deficiency compared with nonvegetarian women. Dietary intake of ASFs was associated with vitamin B-12 status: women who had vitamin B-12 deficiency had significantly lower consumption of ASFs, including poultry ($P = 0.006$) and other meat ($P = 0.001$), but not milk, eggs, or fish ($P > 0.05$). Findings are consistent with previous research that identified vegetarianism and low ASF intake as risk factors for vitamin B-12 deficiency (as dietary vitamin B-12 is found exclusively in ASFs) (44) and other studies in India, which reported associations between ASF intake and vitamin

B-12 status, including among children (83) and pregnant women (84). In contrast, in a study in rural Nagpur with a lower prevalence of vitamin B-12 deficiency (31%) and higher prevalence of vegetarianism (28%), self-reported vegetarian diet was not significantly associated with vitamin B-12 concentrations (63). Low dietary intake of ASFs may be due to limited availability or access in the food supply, poverty, or other socioeconomic, cultural, religious, or personal factors (44). Low ASF intake is a risk factor for vitamin B-12 deficiency, in addition to other risk factors such as poor bioavailability, pernicious anemia (an autoimmune disease), gastrointestinal infections, and certain medications (e.g., metformin) (44, 85).

In the current study, we noted a high prevalence of vitamin B-12 deficiency (48.3%) in combination with a low prevalence of self-reported vegan/vegetarianism (7%). Findings highlight the low frequency of ASF intake even among nonvegetarians in this population. Although this finding may be unexpected in other settings, it is consistent with national and regional data in India. While ∼30% of women aged 15–49 y in NFHS-4 reported being vegetarian (i.e., never consuming fish, chicken, or meat), among nonvegetarians, ∼27% reported consuming fish, chicken, or meat occasionally; 37% reported consuming weekly; and only 1% reported daily consumption (86). Similarly, in a study among pregnant women in Uttar Pradesh, India (87), the prevalence of self-reported lacto-vegetarians was high (46.4%); however, among nonvegetarians, only 8.0% and 4.0% reported consuming flesh foods and eggs, respectively, in the past 24 h. This study found that dairy consumption accounted for 99.6% and 89.6% of vitamin B-12 intake for lacto-vegetarians and nonvegetarians, respectively. Although vitamin B-12 intake was significantly higher in the nonvegetarian group [median (IQR): 0.6 μg (0.2, 1.4) vs. 0.3 μg (0.1, 1.0)], the absolute intake for both groups was considerably lower than the US or Indian Recommended Dietary Allowance (77). In the current study, the low frequency of ASF intake (even among nonvegetarians) and lack of vitamin B-12 supplementation or fortification may explain the high prevalence of vitamin B-12 deficiency in this population.

While RBC folate concentrations are directly linked to NTD risk and represent an average of the last 120 d of folate intake, serum folate reflects recent intake. In the current study, the prevalence of serum folate deficiency (<7.0 nmol/L) was 3.5%. Previous studies in India have evaluated serum folate, with the prevalence of deficiency ranging from 0% to 54% (57, 63, 65). In a recent systematic review of folate status in WRA (12–49 y) globally, the prevalence of serum folate deficiency (39 surveys) ranged from <1% to 79% (46). However, serum folate was evaluated using a variety of laboratory methods and cutoffs, data included both pregnant and nonpregnant women, and did not report lactation status. To date, few national surveys have evaluated serum folate in nonpregnant WRA using the microbiological assay (MBA) (46).

Although the prevalence of RBC folate deficiency (<305 nmol/L) was 7.6% in the current study, 79.3% of WRA had RBC folate <748 nmol/L, the threshold for optimal NTD prevention. In the current study, the prevalence of RBC folate insufficiency was higher among WRA who lived in rural areas compared with those in urban settings, and among WRA who were younger, not married, and who had not previously given birth. There were no differences in the prevalence of RBC folate insufficiency by self-reported dietary patterns. Globally, few studies have evaluated RBC folate status, including nonpregnant WRA, and there are limited population-based data using the MBA. In the systematic review of folate status in WRA globally, 18 surveys assessed RBC folate, with deficiency ranging from ∼0% (<305 nmol/L, protein-binding assay, Canada) (88) to 49% (<342 nmol/L, MBA with folic acid calibrator, Kyrgyzstan) (46). In 10 of these surveys, RBC folate was evaluated using an MBA; however, surveys included pregnant and nonpregnant women and did not report lactation status, which constrains comparability of findings (46).

The RBC folate distribution in this population was used to estimate the prevalence of folate-sensitive NTDs, as has been done in previous studies of populations in the United States and Guatemala (37, 89, 90). The estimated NTD risk in this population (20.6 NTDs per 10,000 live births) was considerably higher than in the United States—as estimated with RBC folate concentrations (7.3 NTDs per 10,000 live births; 95% UI: 5.5–9.4) or through high-quality NTD surveillance (7 NTDs per 10,000 live births)—where moderate–low-dosage folic acid fortification is mandatory (89, 91). Estimates from the current study are also higher than in a Guatemalan population (14 NTDs per 10,000 live births; 95% UI: 11.1–18.6) but similar to one region of Guatemala likely unreached by fortification (Norte region: 26 NTDS per 10,000 births) (90). The rural/urban differences in RBC folate concentrations in the current study were moderate and did not result in substantial differences between estimated NTD prevalence. Findings suggest an NTD risk more than twice as high as observed in populations with mandatory folic acid fortification programs.

The current study is a population-based biomarker survey among WRA—among the largest of its kind to date—and is the first to use the folate microbiological assay in southern India. There was a high prevalence of concurrent vitamin B-12 and RBC folate insufficiency (63.7% both vitamin B-12 and RBC folate insufficiency; 90.1% vitamin B-12 or RBC folate insufficiency). Due to the interrelated metabolism of vitamin B-12 and folate and their roles in the development of anemia and NTDs (38, 51), assessment of the dual burden of vitamin B-12 and folate insufficiencies is of particular importance for WRA.

This study has several limitations. The cross-sectional design does not enable evaluation of changes in biomarkers over time or determine effects of micronutrients on anemia. This study included WRA who are not currently pregnant or lactating but was not limited to women planning to become pregnant; this constrains evaluation of micronutrient status during the preconception, pregnancy, and lactation periods. In addition, the eligibility age range and heterogeneity in WRA definition in the literature limit comparability to some studies. The response rate was ∼54%; although participants in the biomarker survey were similar to women who did not participate, they may differ on unmeasured confounders; this response rate is consistent with other recent population-based biomarker surveys (e.g., NHANES) (92). In this study, vitamin B-12 status was assessed via total vitamin B-12; inclusion of other circulating and functional (e.g., holo-transcobalamin, methylmalonic acid) biomarkers would improve assessment of vitamin B-12 status.

In summary, in this population-based biomarker survey of WRA, there was a high prevalence of anemia, vitamin B-12 deficiency, and RBC folate insufficiency. The substantial burden of anemia and micronutrient deficiencies in WRA in this setting suggests an opportunity for screening and prevention. Findings provide critical preintervention biomarker data that will directly inform the development (i.e., micronutrient concentrations for salt formulation, dose, power calculations) of

a randomized efficacy trial of quadruple-fortified salt for the prevention of anemia and birth defects in southern India.

Acknowledgments

The authors are grateful to Dr. Anura Kurpad, who served as co-principal investigator at St John's Research Institute in Bangalore, India. The authors' responsibilities were as follows—JLF: designed the research and wrote the initial draft manuscript, with feedback from the study collaborators, and had primary responsibility for final content; AF, HMG, and KSC: revised the manuscript; CBJ and AF: supervised data collection and field activities, under the guidance of the investigators; BB: conducted the laboratory analyses with expert guidance on the folate microbiological assay from SJ, MZ, and CMP; AF and KSC: conducted data analyses; and all authors: contributed to the development of the manuscript, provided feedback, and read and approved the final manuscript.

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