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Original article

Iron status and inflammation in women of reproductive age: A population-based biomarker survey and clinical study☆

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SUMMARY

Background: Women of reproductive age (WRA) are at increased risk for anemia and iron deficiency. However, there is limited population-level data in India, which could help inform evidence-based recommendations and policy.
Aims: To conduct a population-based biomarker survey of anemia, iron deficiency, and inflammation in WRA in Southern India.
Methods: Participants were WRA (15-40 y) who were not pregnant or lactating. Blood samples (n = 979) were collected and analyzed for hemoglobin (Hb), serum ferritin (SF), soluble transferrin receptor (sTfR), C-reactive protein (CRP), and alpha-1 acid glycoprotein (AGP). Anemia and severe anemia were defined as Hb < 12.0 and < 8.0 g/dL. Serum ferritin was adjusted for inflammation using BRINDA methods. Iron deficiency was defined as SF <15.0 μg/L, iron insufficiency was defined as SF < 20.0 and < 25.0 μg/L, and iron deficiency anemia was defined as Hb < 12.0 g/dL and SF < 15.0 μg/L. Inflammation was defined as CRP > 5.0 mg/L or AGP > 1.0 g/L. Restricted cubic spline regression models were also used to determine if alternative SF thresholds should be used t to classify iron deficiency.
Results: A total of 41.5% of WRA had anemia, and 3.0% had severe anemia. Findings from spline analyses suggested a SF cut-off of < 15.0 μg/L, consistent with conventional cut-offs for iron deficiency. 46.3% of WRA had SF < 15.0 μg/L (BRINDA-adjusted: 61.5%), 55.0% had SF < 20.0 μg/L (72.7%), 61.8% had SF < 25.0 μg/L (81.0%), and 30.0% had IDA (34.5%). 17.3% of WRA had CRP > 5.0 mg/L and 22.2% had AGP > 1.0 g/L. The prevalence of ID (rural vs. urban: 49.1% vs. 34.9%; p = 0.0004), iron insufficiency (57.8% vs. 43.8%; p = 0.0005), and IDA (31.8% vs. 22.4%; p = 0.01) were significantly higher in rural areas, although CRP levels were lower and there were no differences in elevated CRP or AGP.
Conclusions: The burden of anemia and iron deficiency in this population was substantial, and increased after adjusting for inflammation, suggesting potential to benefit from screening and interventions.
Registration number: NCT04048330.

1. Introduction

Anemia is a major public health problem globally, affecting over 1.9 billion people [1,2]. The prevalence of anemia is highest in low- and middle-income settings, particularly among women of reproductive age (WRA) and young children [1–4]. Iron deficiency is the

☆ **Data sharing:** Data will be made available upon request to the corresponding author.

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Abbreviations

AGP	alpha-1 acid glycoprotein
AMC	Arogyavaram Medical Centre
BMI	body mass index
CDC	Centers for Disease Control and Prevention
CRP	C-reactive protein
DRDC	Disability Research and Dissemination Center
GM	geometric mean
Hb	hemoglobin
HMSC	Health Ministry Screening Committee
ICMR	Indian Council of Medical Research
ID	Iron Deficiency
IEC	Institutional Ethics Committee
INR	Indian rupees
IRB	Institutional Review Board
K2EDTA	dipotassium ethylenediaminetetraacetic acid
LOD	limit of detection
SF	serum ferritin
sTfR	soluble transferrin receptor
TBI	total body iron
USD	United States Dollar
WRA	women of reproductive age
95% CI	95% confidence interval

most common micronutrient deficiency globally [5–9] and is the leading cause of anemia [3,9–12], accounting for an estimated 25–50% of anemia cases [3,9,11]. Even mild iron deficiency can impair cognitive function [13–18] and physical work capacity [13,19,20].

Women of reproductive age are at high risk for anemia and iron deficiency, due in part to blood losses during menstruation and increased iron requirements during pregnancy [21–23]. Low iron status in pregnancy has been associated with an increased risk of adverse outcomes for the mother and infant [24–35], including maternal and infant mortality [25], preterm delivery [25,26,33], low birth weight [25–27,33,35], and impaired neurodevelopment in offspring [28,34].

World Health Organization (WHO) recommendations for biomarkers for iron assessment of populations include hemoglobin (Hb), serum ferritin (SF), soluble transferrin receptor (sTfR), and at least one acute phase protein, such as C-reactive protein (CRP) or alpha-1 acid glycoprotein (AGP) [36]. However, hemoglobin is often monitored as the only proxy indicator for iron deficiency [21,37]. For example, in India, in the National Family Health Survey (NFHS), iron status assessment is limited to hemoglobin concentrations (capillary blood samples); NFHS does not include additional biomarkers of iron status. The WHO recently updated hemoglobin thresholds for assessment of anemia [38–42], serum/plasma ferritin cut-offs to define iron status in different population groups [36,43,44], and thresholds for iron status in the context of infection and inflammation [36,45]; and harmonization of different laboratory methods for iron assessment [36,46] and methods for adjusting iron biomarkers for inflammation [36,47]. Differences in methods for hemoglobin assessment (e.g., capillary vs. venous blood samples, portable hemoglobinometer vs. automated hematology analyzers) constrain direct comparison of estimates [48,49].

There is limited representative population-based data on iron status – particularly in resource-limited settings with the highest anticipated burden of iron deficiency. The World Health Organization recommends evaluation of inflammatory biomarkers – i.e., C-reactive protein and/or alpha-1 acid glycoprotein – in iron status assessment at the population level [83]. However, few national or population-representative surveys monitor data on iron biomarkers beyond hemoglobin [21,37]. Accurate assessment of iron status, including inflammatory biomarkers, is critical to characterize the burden of iron deficiency at the population level and inform evidence-based recommendations and policy, particularly in settings such as India where the burden of anemia and iron deficiency is estimated to be among the highest in the world [50].

The objective of this study was to conduct a biomarker survey of iron status in non-pregnant, non-lactating WRA living in Chittoor district, Andhra Pradesh, in India. A recent analysis of NHANES described methods for physiological determination of serum ferritin cut-offs to classify iron deficiency in the U.S. population for non-pregnant women, and suggested findings needed to be replicated in other populations in international settings with a high burden of iron deficiency [51]. To our knowledge, this is the first study to use this approach to evaluate physiologically based serum ferritin thresholds for iron deficiency in a population representative sample internationally. Findings will provide population-representative iron biomarker data in WRA and inform a randomized trial of quadruple-fortified salt in this population.

2. Subjects and methods

2.1. Study design

A population-based biomarker survey was conducted as part of a periconceptional surveillance program in Chittoor district, Andhra Pradesh, in India. The study design (NCT04048330) [52] and findings for primary outcomes (i.e., vitamin B_{12} and folate status, hemoglobin and anemia) have been previously described [52,53]. Briefly, a census of all households within the 50-km^2 catchment area of Arogyavaram Medical Centre (AMC) was conducted (n = 6552; rural: 3124; urban: 3428). Rural and urban areas were defined based on the Government of India classifications for Census tract (i.e., constituents of urban areas are Statutory towns, Census towns, and Outgrowths). All rural households and a simple random sample of urban households (n = 1000) were revisited to confirm household member rosters and evaluate the eligibility of individuals in the households for the biomarker survey.

2.2. Study population

WRA were eligible for participation in the biomarker survey if they were 15–40 years of age and were not pregnant or lactating; there were no other exclusion criteria. An *a priori* algorithm was used to identify one eligible woman per household; however, programmatically, all WRA were invited to participate and research staff were blinded to the identity of the selected woman. Women who reported that they were currently pregnant or lactating, or who had severe anemia (Hb < 8.0 g/dL) [54] were referred to a local clinic for follow up.

2.3. Informed consent

Informed consent (≥ 18 y) or assent (15 to < 18 y) was obtained from all study participants prior to the start of data collection, with audio-visual recording as per guidelines of the government of India. If an individual was not able to read, the form was read to her in the presence of a literate witness who signed the form, and the woman affixed her thumbprint on the form.

2.4. Ethics

The study protocol was reviewed and approved by the Institutional Review Board (IRB) at Cornell University and the Institutional Ethics Committees (IEC) at AMC and St. John's Research Institute. The protocol was reviewed in accordance with the Centers for Disease Control and Prevention (CDC) human research protection procedures and was determined to be a non-research, routine surveillance activity. A non-disclosure agreement for personally identifiable information and a data sharing agreement for de-identified data were established. This study received clearance from the Indian Council of Medical Research (ICMR) Health Ministry Screening Committee (HMSC). The study protocol was registered at ClincialTrials.gov (NCT04048330). Findings from this biomarker survey are directly informing a randomized trial of quadruple-fortified salt (NCT03853304) in WRA in Southern India.

2.5. Data collection

Data was collected electronically on tablets at AMC; data collection procedures have been previously described [52,55]. After verification of eligibility, socio-demographic, dietary (interviewer-administered 24-h recall and additional questions on consumption of micronutrient supplementation, animal-source foods (including type and frequency), and fortified food products), anthropometric, and clinical data, and biological specimens (blood, saliva, urine) were collected.

2.6. Laboratory analyses

Biological samples were collected, processed, and stored using standardized laboratory protocols [52]. Venous blood samples (12-mL) were collected in 3 vacutainers (i.e., red-top, purple-top dipotassium ethylenediaminetetraacetic acid (K2EDTA), and blue-top metal-free K2EDTA; BD Biosciences) and stored in a portable freezer unit that was set to 4–6 °C (i.e., optimal refrigeration temperature) until processing < 4 h after collection. Complete blood count was analyzed *via* automated Coulter counter (Coulter HMX). Iron and inflammatory biomarkers were analyzed in batch at St. John's Research Institute in Bangalore, India, after completion of data collection. SF was measured by electrochemiluminescence (E411, Roche Diagnostics Mannheim, USA). sTfR, CRP, and AGP concentrations were analyzed *via* the Roche COBAS Integra 400 plus analyzer (Roche Diagnostics, Germany).

2.7. Definitions of outcomes

Anemia was defined as Hb < 12.0 g/dL, and severe anemia was defined as Hb < 8.0 g/dL [54]. Iron deficiency was defined as SF < 15.0 μg/L and iron insufficiency was defined as SF < 20.0 μg/L and SF <25.0 μg/L [36,51]. Iron deficiency anemia (IDA) was defined as Hb < 12.0 g/dL and SF < 15.0 μg/L [36,54]. Elevated inflammatory biomarkers were defined as CRP >5.0 mg/L or AGP >1.0 g/L [36]. Total body iron (TBI) was estimated using the method proposed by Cook [56]:

$$\text{TBI (mg/kg)} = -[\log_{10}(\text{sTfR(mg/L) X 1000/SF(μg/L)}) - 2.8229] / 0.1207$$

Iron deficiency was defined as SF < 15.0 μg/L in primary analyses and as TBI < 0.0 mg/kg and sTfR > 8.3 mg/L in additional analyses [36,56–59]. Women's dietary patterns were defined as vegan (i.e., no consumption of animal source foods, including dairy, eggs, fish, poultry, or meats), ovo-vegetarian (egg consumption), lacto-vegetarian (dairy consumption), lacto-ovo-vegetarian (dairy and egg consumption), pesco-vegetarian (fish consumption), pollo-vegetarian (poultry consumption) or non-vegetarian (meat consumption), and data was recorded on type and frequency of animal source food consumption.

2.8. Statistical analyses

Hemoglobin was adjusted for self-reported smoking status in accordance with WHO guidelines [54]. Serum ferritin concentrations were adjusted for inflammation using the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) methods [45,60] with distributions in this population as reference. Adjustment using the Thurnham method [61,62] was also included for comparison. Continuous biomarker and household income variables were natural logarithmically transformed for analyses. For biomarker analyses, values that were outside of the assay limits of detection (LOD) were set to half the LOD (if below the lower LOD), or 2-times the LOQ (if above the upper LOQ). Geometric means (GM) and 95% confidence intervals (95% CI) were calculated to facilitate statistical inference. TBI was presented as arithmetic mean (95% CI). Restricted cubic splines were used to examine distributions of hemoglobin and sTfR with serum ferritin to determine physiological and population-based serum ferritin thresholds for iron-deficient erythropoiesis. For this analysis, observations were excluded for missing Hb assessments, serum ferritin > 150.0 μg/L, CRP > 5.0 mg/L, or white blood count > 10,000/μL [51].

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 to the overall surveillance population and were used to calculate overall weighted population characteristics. Weighted and unweighted results for sociodemographic characteristics and iron status of the population are provided in Table 1 and Table 2, respectively. For interpretation purposes, results were stratified by rural/urban strata and unweighted results are reported in the text. Chi-square tests and one-way ANOVA were used to evaluate differences in categorial and continuous variables, respectively, and p-values less than 0.05 were considered significant. Statistical analyses were conducted using SAS 9.4 (SAS Institute, Inc., Cary, NC, USA). The analyses were reproduced by the Cornell Results Reproduction (R-squared) at the Cornell Institute for Social and Economic Research (CISER).

3. Results

Characteristics of participants in this study are presented in Table 1, and a flow chart of households and participants is presented in Fig. 1. On average, participants were 28.8 years old; most (83.8%) WRA had some formal education and were currently married (79.4%). Among married participants, 99.2% reported having children and 83.3% reported having 2 or more children. Most sociodemographic characteristics were similar between WRA living in rural and urban households (e.g., age, household size, marital status, education, parity). Among adult women (≥ 18 y), 23.4% were overweight (BMI ≥ 25.0 to < 30.0 kg/m^2), and 9.6% were obese (BMI ≥ 30.0 kg/m^2). The majority of WRA (93.1%) reported following a non-vegetarian diet (e.g., consumption of meat, poultry, or fish), although consumption of animal source foods was low. Although 67.2% of women consumed dairy (curd, milk, ghee, or cheese) at least once per week, less than 25% of women reported consuming eggs or other animal source foods (e.g., poultry, meat, fish) more than once a week. Women living in rural households were less likely to be non-vegetarian (p =0.0001), and reported less frequent

Table 1
Characteristics of the study population.

Variables	n	Total, Weighted	Total (n = 980), Unweighted	Rural (n = 788), Unweighted	Urban (n = 192), Unweighted	P-value[e]
		GM (95% CI) or %	GM (95% CI) or n (%)	GM (95% CI) or n (%)	GM (95% CI) or n (%)	
Sociodemographic						
Age, y	980	28.9 (28.4, 29.5)	28.8 (28.4, 29.3)	28.8 (28.3, 29.3)	29.1 (28.1, 30.2)	0.57
15 to <18		5.0	49 (5.0)	41 (5.2)	8 (4.2)	0.81
18 to <26		23.4	229 (23.4)	187 (23.7)	42 (21.9)	
26 to <36		46.1	453 (46.2)	359 (45.6)	94 (49.0)	
36 to 40		25.4	249 (25.4)	201 (25.5)	48 (25.0)	
Highest level of education completed	975					0.65
No formal schooling		16.2	158 (16.2)	125 (15.9)	33 (17.3)	
Grades 1-5		17.8	174 (17.8)	138 (17.6)	36 (18.8)	
Grades 6-8		18.1	177 (18.2)	142 (18.1)	35 (18.3)	
Grades 9-10		21.2	207 (21.2)	165 (21.0)	42 (22.0)	
Grades 11-12		10.8	106 (10.9)	83 (10.6)	23 (12.0)	
College or graduate degree		15.8	153 (15.7)	131 (16.7)	22 (11.5)	
Marital status	975					0.56
Currently married		79.3	774 (79.4)	617 (78.7)	157 (82.2)	
Widowed, divorced, separated		3.6	35 (3.6)	29 (3.7)	6 (3.1)	
Never married		17.1	166 (17.0)	138 (17.6)	28 (14.7)	
Parity	975	1.7 (1.6, 1.8)	1.7 (1.6, 1.8)	1.7 (1.6, 1.8)	1.6 (1.5, 1.8)	0.67
Nulliparous		23.7	231 (23.7)	187 (23.9)	44 (23.0)	0.48
Primiparous		9.2	90 (9.2)	68 (8.7)	22 (11.5)	
Multiparous		67.1	654 (67.1)	529 (67.5)	125 (65.4)	
Currently has children	743	98.8	737 (99.2)	592 (99.3)	145 (98.6)	0.40
Number of children		2.0 (1.9, 2.1)	2.0 (1.9, 2.1)	2.0 (1.9, 2.1)	1.9 (1.7, 2.2)	0.57
Anthropometric						
Weight, kg	969	53.5 (52.5, 54.4)	53.0 (52.2, 53.7)	52.6 (51.7, 53.4)	54.6 (52.8, 56.4)	0.06
Height, cm	969	153.1 (152.6, 153.5)	153.2 (152.9, 153.6)	153.4 (153.0, 153.8)	152.7 (151.9, 153.5)	0.14
Body mass index, kg/m^2	969	22.8 (22.5, 23.2)	22.6 (22.2, 22.9)	22.3 (22.0, 22.7)	23.4 (22.7, 24.2)	0.01
Body mass index[a,b]	920					
<18.5		19.4	177 (19.2)	153 (20.8)	24 (13.1)	0.02
18.5 to <25.0		47.9	440 (47.8)	354 (48.0)	86 (47.0)	
25.0 to <30.0		23.1	215 (23.4)	159 (21.6)	56 (30.6)	
≥30.0		9.6	88 (9.6)	71 (9.6)	17 (9.3)	
Body mass index[a,c]	920					
<18.5		19.4	177 (19.2)	153 (20.8)	24 (13.1)	0.04
18.5 to <23.0		33.5	308 (33.5)	250 (33.9)	58 (31.7)	
23.0 to <27.5		27.4	253 (27.5)	198 (26.9)	55 (30.1)	
≥27.5		19.6	182 (19.8)	136 (18.5)	46 (25.1)	
Mid-upper arm circumference, cm	969	26.9 (26.6, 27.2)	26.7 (26.5, 27.0)	26.6 (26.3, 26.9)	27.3 (26.7, 27.9)	0.03
Waist circumference, cm	969	75.1 (74.3, 76.0)	74.6 (73.9, 75.3)	74.2 (73.4, 75.0)	76.3 (74.7, 78.0)	0.03
>88.9[a] cm	920	13.3	123 (13.4)	93 (12.6)	30 (16.4)	0.18
Dietary						
Dietary preference[d]	974					0.002
Vegan		0.3	3 (0.3)	3 (0.4)	0 (0.0)	
Lacto-vegetarian		3.7	35 (3.6)	35 (4.5)	0 (0.0)	
Ovo-vegetarian		0.1	1 (0.1)	1 (0.1)	0 (0.0)	
Lacto-ovo vegetarian		3.0	28 (2.9)	27 (3.4)	1 (0.5)	
Pesco-vegetarian		0.3	3 (0.3)	3 (0.4)	0 (0.0)	
Pollo-vegetarian		15.4	149 (15.3)	128 (16.3)	21 (11.0)	
Non-vegetarian		77.1	755 (77.5)	586 (74.8)	169 (88.5)	
Dairy Consumption	974					0.02
Never		5.4	53 (5.4)	42 (5.4)	11 (5.8)	
Almost never (<1x per month)		3.7	35 (3.6)	35 (4.5)	0 (0.0)	
Occasionally (<1x/week)		8.1	79 (8.1)	63 (8.0)	16 (8.4)	
Often (about 1x/week)		15.5	152 (15.6)	114 (14.6)	38 (19.9)	
Very often (>1x/week)		67.3	655 (67.2)	529 (67.6)	126 (66.0)	
Egg Consumption	969					0.003
Never		7.6	72 (7.4)	65 (8.4)	7 (3.7)	
Almost never (<1x per month)		11.2	107 (11.0)	95 (12.2)	12 (6.3)	
Occasionally (<1x/week)		18.2	178 (18.4)	134 (17.2)	44 (23.0)	
Often (about 1x/week)		38.6	376 (38.8)	289 (37.1)	87 (45.5)	
Very often (>1x/week)		24.5	236 (24.4)	195 (25.1)	41 (21.5)	
Poultry Consumption	973					0.0008
Never		11.1	106 (10.9)	99 (12.7)	7 (3.7)	
Almost never (<1x per month)		8.4	81 (8.3)	67 (8.6)	14 (7.3)	
Occasionally (<1x/week)		12.3	119 (12.2)	100 (12.8)	19 (9.9)	
Often (about 1x/week)		54.8	537 (55.2)	409 (52.3)	128 (67.0)	
Very often (>1x/week)		13.4	130 (13.4)	107 (13.7)	23 (12.0)	
Meat Consumption	973					<0.0001
Never		22.9	219 (22.5)	197 (25.2)	22 (11.5)	
Almost never (<1x per month)		31.1	303 (31.1)	240 (30.7)	63 (33.0)	
Occasionally (<1x/week)		21.5	213 (21.9)	148 (18.9)	65 (34.0)	
Often (about 1x/week)		21.5	209 (21.5)	169 (21.6)	40 (20.9)	

Table 1 (*continued*)

Variables	n	Total, Weighted	Total (n = 980), Unweighted	Rural (n = 788), Unweighted	Urban (n = 192), Unweighted	P-value[e]
		GM (95% CI) or %	GM (95% CI) or n (%)	GM (95% CI) or n (%)	GM (95% CI) or n (%)	
Very often (>1x/week)		3.1	29 (3.0)	28 (3.6)	1 (0.5)	
Fish Consumption	973					0.04
Never		31.9	308 (31.7)	261 (33.4)	47 (24.6)	
Almost never (<1x per month)		39.6	388 (39.9)	298 (38.1)	90 (47.1)	
Occasionally (<1x/week)		18.2	178 (18.3)	139 (17.8)	39 (20.4)	
Often (about 1x/week)		7.6	74 (7.6)	61 (7.8)	13 (6.8)	
Very often (>1x/week)		2.6	25 (2.6)	23 (2.9)	2 (1.0)	

Abbreviations: GM: geometric mean; INR: Indian rupees; USD: United States Dollar, 95% CI: 95% confidence interval.

[a] Among participants ≥ 18 years old (n = 931; rural: 747; urban: 184).

[b] BMI categories as defined by the WHO [84].

[c] BMI categories for Asian populations [85].

[d] Vegan: did not consume any animal source foods; Lacto-vegetarian: dairy consumption; Ovo-vegetarian: egg consumption; Lacto-Ovo vegetarian: dairy and egg consumption; Pesco-vegetarian: fish consumption; Pollo-vegetarian: poultry consumption.

[e] Chi-square statistics and one-way ANOVA were used to evaluate differences in continuous and categorical 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).

consumption of animal source foods, compared to WRA living in urban households (Table 1).

Iron status of WRA is presented in Table 2. A total of 41.5% of WRA were anemic (Hb < 12.0 g/dL) and 3.0% had severe anemia (Hb < 8.0 g/dL). Mean serum ferritin concentrations were 16.8 μg/L (95% CI: 15.8, 17.9); 40.6% had SF < 12.0 μg/L, 46.3% of WRA were iron deficient (SF < 15.0 μg/L), and 55.0% had iron insufficiency (SF < 20.0 μg/L: 55.0%; SF < 25.0 μg/L: 61.8%). Findings from the spline-based methods resulted in a suggested a SF threshold of < 15.0 μg/L (Supplemental Figure I and Figure II), consistent with conventional cut-offs for iron deficiency A total of 30.0% of WRA had IDA (SF < 15.0 μg/L and Hb < 12.0 g/dL). The prevalence of elevated sTfR (> 8.3 mg/L) was 15.4%, and 29.4% of women had TBI < 0.0 mg/kg. After weighting results for study design, SF concentrations increased (GM: 18.4 [95% CI: 17.1, 19.8] μg/L); however, other continuous iron biomarkers (sTfR, TBI) and the prevalence of iron deficiency, iron insufficiency, and IDA were unchanged. The prevalence of elevated inflammatory biomarkers, CRP and AGP, are presented in Table 2. A total of 17.3% of WRA had CRP > 5.0 mg/L, 29.9% had CRP > 3.0 mg/L, and 56.5% had CRP > 1.0 mg/L (GM: 1.2 [95% CI: 1.1, 1.4] mg/L); 22.2% of women had AGP > 1.0 g/L (GM: 0.8 [95% CI: 0.8, 0.8] g/L), and 27.9% had either elevated CRP (> 5.0 mg/L) or AGP (> 1.0 g/L). In terms of Thurnham's stages of inflammation, 5.6% of WRA had elevated CRP only (incubation: CRP > 5.0 mg/L and AGP ≤ 1.0 g/L), 11.7% were in early convalescence (CRP >5.0 mg/L and AGP >1.0 g/L), 10.6% were in late convalescence (CRP ≤5.0 mg/L and AGP >1.0 g/L), and 72.1% had both CRP and AGP levels within the reference ranges (CRP ≤5.0 mg/L and AGP ≤1.0 g/L).

Serum ferritin concentrations adjusted for inflammation using BRINDA and Thurnham methods are presented in Table 2. After adjusting SF for inflammation using BRINDA methods, the prevalence of iron deficiency was 61.5% (SF < 15.0 μg/L), and 72.7% of women had iron insufficiency (SF < 20.0 μg/L); 54.2% had SF < 12.0 μg/L, 34.5% had IDA, and 38.8% had TBI <0.0 mg/kg. After adjusting SF for inflammation using Thurnham methods, 49.6% of women had iron deficiency, 58.8% had iron insufficiency, 43.6% had SF < 12.0 μg/L, 31.0% had IDA, and 31.7% had TBI <0.0 mg/kg. After weighting results for study design, SF levels increased (BRINDA GM: 11.3 [95% CI: 10.5, 12.1] μg/L; Thurnham GM: 16.3 [95% CI: 15.2, 17.5] μg/L), while other iron biomarkers (sTfR, TBI) and the prevalence of iron deficiency, iron insufficiency, and IDA remained similar to unweighted results.

Anemia and iron status stratified by rural or urban residence are also presented in Table 2. Hemoglobin concentrations (rural: GM 11.8 [95% CI: 11.7, 11.9] vs. urban: GM 12.1 [95% CI: 11.8, 12.4] g/dL; p =0.07) and the prevalence of anemia (Hb < 12.0 g/dL; rural: 42.2% vs. urban: 38.5%; p = 0.36) or severe anemia (Hb < 8.0 g/dL; rural: 3.4% vs. urban: 1.0%; p = 0.08) were not significantly different between rural and urban areas. However, WRA residing in rural households had significantly lower iron status for all other biomarkers, including SF (rural: GM 15.7 [95% CI: 14.7, 16.9] vs. urban: GM 22.2 [95% CI: 19.3, 25.5] μg/L; p < 0.0001), sTfR (rural: GM 5.0 [95% CI: 4.8, 5.2] vs. urban: 4.5 [95% CI: 4.2, 4.8] mg/L; p = 0.008), and TBI (rural: mean 2.6 [95% CI: 2.3, 3.0) vs. urban: mean 4.3 [95% CI: 3.6, 5.0] mg/kg; p < 0.0001), compared to WRA in urban households. The prevalence of iron deficiency (SF <15.0 μg/L; rural: 49.1% vs. urban: 34.9%; p = 0.0004), iron insufficiency (SF < 20.0 μg/L; rural: 57.8% vs. urban: 43.8%; p = 0.0005), and IDA (SF < 15.0 and Hb < 12.0 g/dL; rural: 31.8% vs. urban: 22.4%; p = 0.01) were significantly higher in WRA residing in rural households compared to urban areas. Similarly, the prevalence of iron deficiency, defined as elevated sTfR (sTfR > 8.3 mg/L; rural: 16.7% vs. urban: 9.9%; p = 0.02) or low TBI (TBI < 0.0 mg/kg; rural: 31.7% vs. urban: 19.9%; p = 0.001), was significantly higher in WRA in rural areas, compared to urban households.

The prevalence of inflammation, defined as CRP > 5.0 mg/L (rural: 16.2% vs. urban: 21.9%; p = 0.06) or AGP > 1.0 g/L (rural: 22.0% vs. urban: 23.0%; p = 0.77) was not significantly different between rural and urban strata. However, women residing in rural households had significantly lower CRP concentrations compared to WRA in the urban area (rural: GM 1.1 [95% CI: 1.0, 1.3] vs. urban: GM 1.7 [95% CI: 1.4, 2.1] mg/L; p = 0.0002). The prevalence of CRP > 3.0 mg/L (rural: 27.0% vs. urban: 41.7%; p = 0.0001) or CRP >1.0 mg/L (rural: 54.1% vs. 66.7%; p = 0.002) was also significantly lower in WRA in rural households, compared to the urban area.

After adjusting SF for inflammation using BRINDA methods, SF concentrations remained significantly lower in rural areas compared to urban settings (rural GM: 10.0 [95% CI: 9.4, 10.7]; urban GM: 12.9 [95% CI: 11.3, 14.8] μg/L; p = 0.0008), and the prevalence of iron deficiency (rural: 64.2% vs. urban: 50.3%; p = 0.0004) was significantly higher in WRA living in rural households. The prevalence of iron insufficiency (rural: 74.0% vs. urban: 67.5%; p = 0.07) and IDA (rural: 35.9% vs. urban: 28.8%; p =0.06) were no longer significantly different between urban and rural strata after adjusting for inflammation. After adjusting for inflammation using Thurnham methods, SF concentrations remained significantly lower in WRA in rural areas compared to urban areas, and the prevalence of iron deficiency, iron insufficiency, and IDA were significantly higher in WRA living in rural households.

Table 2
Iron status and inflammation in women of reproductive age.[a].

	n	Total, Weighted	Total (n = 979), Unweighted	Rural (n = 787), Unweighted	Urban (n = 192), Unweighted	P-value[g]
		GM (95% CI) or %	GM (95% CI) or n (%)	GM (95% CI) or n (%)	GM (95% CI) or n (%)	
Hemoglobin[b], g/dL	979	11.9 (11.8, 12.1)	11.9 (11.7, 12.0)	11.8 (11.7, 11.9)	12.1 (11.8, 12.4)	0.07
<12.0 g/dL		41.6	406 (41.5)	332 (42.2)	74 (38.5)	0.36
<8.0 g/dL		3.0	29 (3.0)	27 (3.4)	2 (1.0)	0.08
Serum ferritin, μg/L	978	18.4 (17.1, 19.8)	16.8 (15.8, 17.9)	15.7 (14.7, 16.9)	22.2 (19.3, 25.5)	<0.0001
<12.0 μg/L		40.9	397 (40.6)	336 (42.7)	61 (31.8)	0.005
<15.0 μg/L		46.7	453 (46.3)	386 (49.1)	67 (34.9)	0.0004
<20.0 μg/L		55.4	538 (55.0)	454 (57.8)	84 (43.8)	0.0005
<25.0 μg/L		62.1	604 (61.8)	507 (64.5)	97 (50.5)	0.0004
Iron deficiency anemia[c]		30.2	293 (30.0)	250 (31.8)	43 (22.4)	0.01
Soluble transferrin receptor, mg/L	976	5.3 (5.1, 5.5)	5.4 (5.3, 5.6)	5.5 (5.4, 5.7)	5.0 (4.7, 5.3)	0.007
>8.6 mg/L		16.6	160 (16.4)	141 (18.0)	19 (9.9)	0.007
Soluble transferrin receptor[d], mg/L	976	4.8 (4.6, 5.0)	4.9 (4.7, 5.1)	5.0 (4.8, 5.2)	4.5 (4.2, 4.8)	0.008
>8.3 mg/L		15.5	150 (15.4)	131 (16.7)	19 (9.9)	0.02
Total body iron[e], mg/kg	976	3.4 (3.0, 3.8)	3.0 (2.7, 3.3)	2.6 (2.3, 3.0)	4.3 (3.6, 5.0)	<0.0001
<0.0 mg/kg		29.7	287 (29.4)	249 (31.7)	38 (19.9)	0.001
C-reactive protein, mg/L	978	1.4 (1.2, 1.5)	1.2 (1.1,1.4)	1.1 (1.0, 1.3)	1.7 (1.4, 2.1)	0.0002
>1.0 mg/L		56.2	553 (56.5)	425 (54.1)	128 (66.7)	0.002
>3.0 mg/L		29.5	292 (29.9)	212 (27.0)	80 (41.7)	0.0001
>5.0 mg/L		17.1	169 (17.3)	127 (16.2)	42 (21.9)	0.06
Alpha-1 acid glycoprotein, g/L	976	0.8 (0.8, 0.8)	0.8 (0.8, 0.8)	0.8 (0.8, 0.8)	0.8 (0.8, 0.9)	0.35
>1.0 g/L		22.2	217 (22.2)	173 (22.0)	44 (23.0)	0.77
CRP>5.0 mg/L or AGP>1.0 g/L	976	27.8	272 (27.9)	212 (27.0)	60 (31.4)	0.22
Serum ferritin (BRINDA-adjusted), μg/L		11.3 (10.5, 12.1)	10.5 (9.9, 11.2)	10.0 (9.4, 10.7)	12.9 (11.3, 14.8)	0.0008
<12.0 μg/L		54.6	529 (54.2)	446 (56.8)	83 (43.5)	0.0009
<15.0 μg/L		61.8	600 (61.5)	504 (64.2)	96 (50.3)	0.0004
<20.0 μg/L		72.9	710 (72.7)	581 (74.0)	129 (67.5)	0.07
<25.0 μg/L		81.2	791 (81.0)	645 (82.2)	146 (76.4)	0.07
Iron deficiency anemia[c]		34.7	337 (34.5)	282 (35.9)	55 (28.8)	0.06
Total body iron[e], mg/kg		1.6 (1.3, 2.0)	1.3 (1.0, 1.6)	1.0 (0.7, 1.4)	2.4 (1.7, 3.0)	0.0008
<0.0 mg/kg		39.2	379 (38.8)	325 (41.1)	54 (28.3)	0.0008
Thurnham stages of inflammation[f]	976					0.20
Incubation		5.5	55 (5.6)	39 (5.0)	16 (8.4)	
Early Convalescence		11.6	114 (11.7)	88 (11.2)	26 (13.6)	
Late Convalescence		10.6	103 (10.6)	85 (10.8)	18 (9.4)	
Healthy		72.2	704 (72.1)	573 (73.0)	131 (68.6)	
Serum ferritin (Thurnham-adjusted), μg/L		16.3 (15.2, 17.5)	15.0 (14.1, 15.9)	14.0 (13.1, 15.0)	19.5 (17.0, 22.4)	<0.0001
<12.0 μg/L		43.9	425 (43.6)	360 (45.9)	65 (34.0)	0.003
<15.0 μg/L		50.0	484 (49.6)	415 (52.9)	69 (36.1)	<0.0001
<20.0 μg/L		59.2	574 (58.8)	486 (61.9)	88 (46.1)	0.0001
<25.0 μg/L		67.8	658 (67.4)	551 (70.2)	107 (56.0)	0.0002
Iron deficiency anemia[c]		31.3	303 (31.0)	259 (33.0)	44 (23.0)	0.008
Total body iron[e], mg/kg		3.0 (2.6, 3.3)	2.5 (2.2, 2.9)	2.2 (1.9, 2.6)	3.8 (3.1, 4.5)	<0.0001
<0.0 mg/kg		32.0	309 (31.7)	267 (34.0)	42 (22.0)	0.001

Abbreviations: AGP: alpha-1 acid glycoprotein; CRP: C-reactive protein, GM: geometric mean, Hb: hemoglobin, TBI: total body iron, 95% CI: 95% confidence interval.

[a] Results outside assay limits of detection were set to either 0.50*LOD (below LOD) or 2*LOD (if above LOD). Results outside assay LODs: soluble transferrin receptor (n = 4 above LOD), CRP (n = 7 below LOD).

[b] Hemoglobin values from complete blood count; adjusted for self-reported smoking status [54].

[c] Serum ferritin <15.0 μg/L and Hb < 12.0 g/dL; hemoglobin adjusted for self-reported smoking status [54].

[d] Ramco; Conversion = Roche-0.299/0.631.

[e] Values are arithmetic mean (95% CI); TBI (mg/kg) = −[$\log_{10}$ (sTfR (mg/L) X 1000/SF(μg/L)) − 2.8229]/0.1207.

[f] Thurnham stages of inflammation: Incubation: CRP > 5.0 mg/L and AGP ≤ 1.0 g/L; Early Convalescence: CRP > 5.0 mg/L and AGP > 1.0 g/L; Late Convalescence: CRP ≤ 5.0 mg/L and AGP > 1.0 g/L; Healthy: CRP ≤ 5.0 mg/L and AGP ≤ 1.0 g/L.

[g] Chi-square statistics and one-way ANOVA were used to evaluate differences in continuous and categorical variables; Hb, SF, sTfR, CRP, AGP were natural logarithmically transformed prior to analyses.

Associations between sociodemographic characteristics and anemia in WRA, stratified by rural and urban residence, are presented in Table 3. The majority of sociodemographic characteristics were not significantly different between anemic and non-anemic women, in rural or urban areas (Table 3).

Sociodemographic characteristics by iron deficiency (i.e., SF < 15.0 μg/L, BRINDA-adjusted) in WRA living in rural and urban households are presented in Table 4. In the rural area, women with iron deficiency were older (p = 0.008), more likely to be married (81.8% vs. 73.2%; p = 0.01) and were more likely to be multiparous (70.7% vs. 62.1%; p = 0.02), compared to WRA who were not iron deficient, after adjusting SF for inflammation using BRINDA methods. In urban households, WRA with iron deficiency were younger (p = 0.04), compared to WRA who were not iron deficient (Table 4). Findings were similar after adjusting SF for inflammation *via* Thurnham methods.

Associations between sociodemographic characteristics and elevated CRP (CRP > 5.0 mg/L) in WRA, stratified by rural and urban residence, are presented in Table 5. In rural areas, WRA with elevated CRP were older (p = 0.0001) compared to WRA without elevated CRP. In urban areas, WRA with elevated CRP were older (p = 0.002), more likely to be married (95.2% vs. 78.5%; p = 0.01), reported increased number of previous pregnancies (p = 0.0004), and were less likely to be nulliparous (4.8% vs. 28.2%; p =0.003), compared to WRA without elevated CRP (Table 5).

Sociodemographic characteristics by other iron biomarkers, including sTfR > 8.3 mg/L and TBI < 0.0 mg/kg, are presented in Supplemental tables (Tables S2 and S3). Most sociodemographic

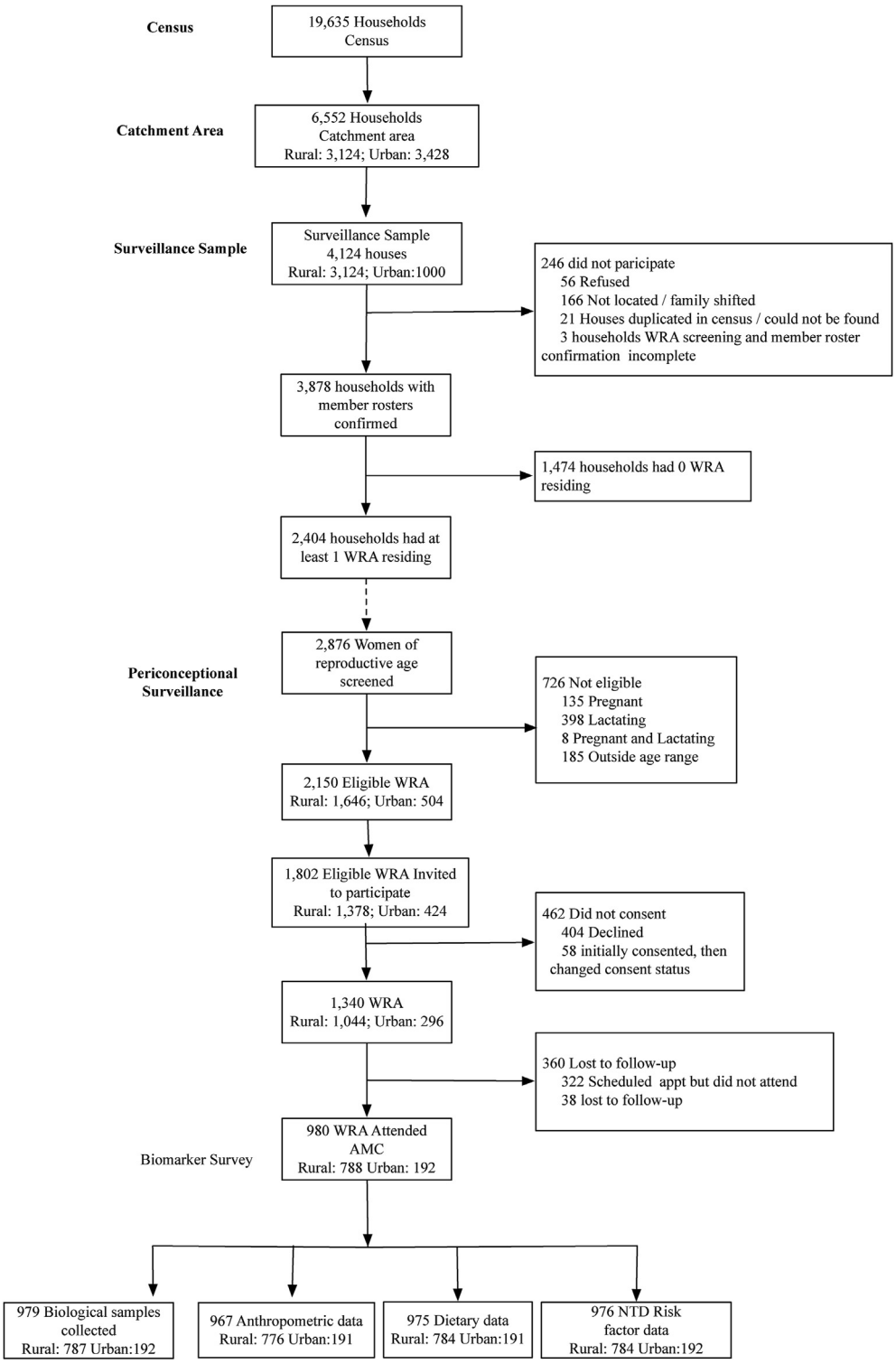

Fig. 1. Participant flow chart.

characteristics, self-reported dietary patterns (vegetarian vs. non-vegetarian) and reported consumption of animal-source foods (i.e., egg, dairy, poultry, meat) were not significantly different between WRA with elevated sTfR and WRA with lower sTfR levels, in the rural or urban areas ($p = 0.003$) (Table S2).

Similarly, among rural WRA, sociodemographic characteristics were not significantly different between WRA with TBI < 0.0 mg/kg and TBI≥ 0.0 mg/kg (Table S3). In urban households, WRA with TBI < 0.0 mg/kg were younger ($p = 0.01$), had more formal educational level ($p = 0.02$), were less likely to be married (60.5% vs. 88.2%; $p = 0.002$), reported fewer previous pregnancies ($p = 0.008$), and were more likely to be nulliparous (39.5% vs. 18.4%; $p = 0.02$) compared to WRA with TBI ≥ 0.0 mg/kg (Table S3).

Table 3
Sociodemographic characteristics of the study population by anemia status.

Variables	n	Rural		P-value[a]	Urban		P-value[a]
		Anemic (n = 332)	Not Anemic (n = 455)		Anemic (n = 74)	Not Anemic (n = 118)	
		GM (95% CI) or n (%)	GM (95% CI) or n (%)		GM (95% CI) or n (%)	GM (95% CI) or n (%)	
Age, y	979	29.0 (28.2, 29.8)	28.6 (27.9, 29.2)	0.45	29.1 (27.5, 30.8)	29.2 (27.9, 30.5)	0.85
15 to <18		13 (3.9)	28 (6.2)	0.49	4 (5.4)	4 (3.4)	0.14
18 to <26		76 (22.9)	111 (24.4)		17 (23.0)	25 (21.2)	
26 to <36		157 (47.3)	202 (44.4)		29 (39.2)	65 (55.1)	
36 to 40		86 (25.9)	114 (25.1)		24 (32.4)	24 (20.3)	
Highest level of education completed	974						
No formal schooling		55 (16.6)	70 (15.5)	0.46	10 (13.7)	23 (19.5)	0.62
Grades 1-5		58 (17.5)	79 (17.5)		12 (16.4)	24 (20.3)	
Grades 6-8		59 (17.8)	83 (18.4)		14 (19.2)	21 (17.8)	
Grades 9-10		78 (23.6)	87 (19.2)		16 (21.9)	26 (22.0)	
Grades 11–12, College/graduate degree		81 (24.5)	133 (29.4)		21 (28.8)	24 (20.3)	
Marital status	974						
Currently married		266 (80.4)	351 (77.7)	0.63	58 (79.5)	99 (83.9)	0.62
Widowed, divorced, living separately		12 (3.6)	17 (3.8)		2 (2.7)	4 (3.4)	
Never married		53 (16.0)	84 (18.6)		13 (17.8)	15 (12.7)	
Parity	974	1.7 (1.6, 1.9)	1.7 (1.5, 1.8)	0.59	1.7 (1.4, 2.0)	1.6 (1.4, 1.9)	0.84
Nulliparous		77 (23.3)	109 (24.1)	0.85	16 (21.9)	28 (23.7)	0.93
Primiparous		27 (8.2)	41 (9.1)		8 (11.0)	14 (11.9)	
Multiparous		227 (68.6)	302 (66.8)		49 (67.1)	76 (64.4)	
Currently has children	743	253 (99.6)	339 (99.1)	0.47	56 (98.2)	89 (98.9)	0.74
Number of children		2.0 (1.8, 2.2)	2.0 (1.9, 2.2)	0.97	1.9 (1.6, 2.3)	1.9 (1.7, 2.3)	0.89

Abbreviations: GM: geometric mean, WRA: women of reproductive age, 95% CI: 95% confidence interval.
[a] Chi-square statistics and one-way 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).

Table 4
Sociodemographic characteristics of the study population by iron deficiency, BRINDA adjusted (SF < 15.0 μg/L)[a].

Variables	n	Rural			Urban		
		Iron Deficient (n = 504)	Not Iron Deficient (n = 281)	P-value[b]	Iron Deficient (n = 96)	Not Iron Deficient (n = 95)	P-value[b]
		GM (95% CI) or n (%)	GM (95% CI) or n (%)		GM (95% CI) or n (%)	GM (95% CI) or n (%)	
Age, y	976	29.3 (28.7, 30.0)	27.8 (27.0, 28.7)	0.008	28.2 (26.9, 29.6)	30.2 (28.8, 31.7)	0.04
15 to <18		23 (4.6)	18 (6.4)		5 (5.2)	3 (3.2)	0.34
18 to <26		106 (21.0)	80 (28.5)		25 (26.0)	16 (16.8)	
26 to <36		243 (48.2)	115 (40.9)		45 (46.9)	49 (51.6)	
36 to 40		132 (26.2)	68 (24.2)		21 (21.9)	27 (28.4)	
Highest level of education completed	971			0.06			0.32
No formal schooling		85 (17.0)	40 (14.3)		13 (13.7)	20 (21.1)	
Grades 1-5		89 (17.8)	47 (16.8)		16 (16.8)	20 (21.1)	
Grades 6-8		92 (18.4)	50 (17.9)		22 (23.2)	13 (13.7)	
Grades 9-10		116 (23.2)	49 (17.5)		19 (20.0)	23 (24.2)	
Grades 11-12		44 (8.8)	39 (13.9)		12 (12.6)	11 (11.6)	
College or graduate degree		75 (15.0)	55 (19.6)		13 (13.7)	8 (8.4)	
Marital status	971			0.01			0.22
Currently married		410 (81.8)	205 (73.2)		74 (77.9)	83 (87.4)	
Widowed, divorced, living separately		18 (3.6)	11 (3.9)		4 (4.2)	2 (2.1)	
Never married		73 (14.6)	64 (22.9)		17 (17.9)	10 (10.5)	
Parity	971	1.8 (1.7, 1.9)	1.5 (1.4, 1.7)	0.008	1.5 (1.3, 1.8)	1.8 (1.5, 2.0)	0.21
Nulliparous		103 (20.6)	82 (29.3)	0.02	25 (26.3)	18 (18.9)	0.46
Primiparous		44 (8.8)	24 (8.6)		11 (11.6)	11 (11.6)	
Multiparous		354 (70.7)	174 (62.1)		59 (62.1)	66 (69.5)	
Currently has children	742	394 (99.2)	197 (99.5)	0.72	68 (97.1)	77 (100.0)	0.14
Number of children		2.0 (1.9, 2.2)	2.0 (1.8, 2.2)	0.89	1.9 (1.6, 2.2)	2.0 (1.7, 2.3)	0.61

Abbreviations: BRINDA: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia, GM: geometric mean, SF: serum ferritin, WRA, women of reproductive age, 95% CI: 95% confidence interval.
[a] Serum Ferritin results missing for n = 1 WRA; Serum Ferritin was not adjusted for n = 2 WRA missing AGP results; results assay limits of detection (LOD) were set to either 0.50*LOD (if below LOD) or 2*LOD (if above LOD). Results outside assay LODs: CRP (n = 7 below LOD).
[b] Chi-square statistics and one-way 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).

Sociodemographic characteristics by elevated AGP levels (AGP > 1.0 g/L), stratified by rural and urban areas are reported in supplemental Table S4. In rural areas, sociodemographic characteristics were not significantly different between WRA with AGP > 1.0 g/L compared to WRA with AGP ≤ 1.0 g/L. In urban areas, WRA with elevated AGP were older (p = 0.04), more likely to be married (95.5% vs. 78.8%; p = 0.01), reported more previous pregnancies (p = 0.02), and were less likely to be nulliparous (6.8% vs. 27.4%; p = 0.01), compared to WRA with AGP ≤1.0 g/L (Table S4).

Table 5
Sociodemographic characteristics of the study population by elevated C-Reactive protein (CRP>5.0 mg/L)[a].

Variables	n	Rural		P-value[b]	Urban		P-value[b]
		CRP>5.0 mg/L (n = 127)	CRP ≤5.0 mg/L (n = 659)		CRP>5.0 mg/L (n = 42)	CRP≤5.0 mg/L (n = 150)	
		GM (95% CI) or n (%)	GM (95% CI) or n (%)		GM (95% CI) or n (%)	GM (95% CI) or n (%)	
Age, y	978	31.1 (29.8, 32.5)	28.3 (27.8, 28.9)	0.0001	32.2 (30.0, 34.6)	28.3 (27.3, 29.4)	0.002
15 to <18		3 (2.4)	38 (5.8)	0.003	0 (0.0)	8 (5.3)	0.10
18 to <26		18 (14.2)	168 (25.5)		5 (11.9)	37 (24.7)	
26 to <36		61 (48.0)	298 (45.2)		24 (57.1)	70 (46.7)	
36 to 40		45 (35.4)	155 (23.5)		13 (31.0)	35 (23.3)	
Highest level of education completed	973			0.47			0.31
No formal schooling		18 (14.4)	107 (16.3)		6 (14.3)	27 (18.1)	
Grades 1-5		26 (20.8)	111 (16.9)		10 (23.8)	26 (17.4)	
Grades 6-8		26 (20.8)	116 (17.7)		11 (26.2)	24 (16.1)	
Grades 9-10		28 (22.4)	137 (20.9)		9 (21.4)	33 (22.1)	
Grades 11–12, college/graduate degree		27 (21.6)	186 (28.3)		6 (14.3)	39 (26.2)	
Marital status	973			0.06			0.01
Currently married		106 (84.8)	510 (77.6)		40 (95.2)	117 (78.5)	
Widowed, divorced, separated		6 (4.8)	23 (3.5)		2 (4.8)	4 (2.7)	
Never married		13 (10.4)	124 (18.9)		0 (0.0)	28 (18.8)	
Parity	973	1.8 (1.5, 2.0)	1.7 (1.6, 1.8)	0.42	2.1 (1.7, 2.6)	1.5 (1.3, 1.7)	0.01
Nulliparous		22 (17.6)	163 (24.8)	0.16	2 (4.8)	42 (28.2)	0.003
Primiparous		14 (11.2)	54 (8.2)		4 (9.5)	18 (12.1)	
Multiparous		89 (71.2)	440 (67.0)		36 (85.7)	89 (59.7)	
Currently has children	743	103 (100.0)	489 (99.2)	0.36	40 (100.0)	105 (98.1)	0.38
Number of children		2.0 (1.7, 2.3)	2.0 (1.9, 2.1)	0.72	2.0 (1.6, 2.5)	1.9 (1.7, 2.2)	0.62

Abbreviations: CRP: C-reactive protein, GM: geometric mean, INR: Indian rupees, USD: United States Dollar, 95% CI: 95% confidence interval.
[a] CRP results missing for n = 1 WRA; Results outside assay limits of detection (LOD) were set to either 0.50*LOD (if below LOD) or 2*LOD (if above LOD). Results outside assay LODs: CRP (n = 7 below LOD).
[b] Chi-square statistics and one-way 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); Values in bold indicate $p < 0.05$ for comparison.

4. Discussion

In this biomarker survey in WRA, there was a high prevalence of anemia and iron deficiency. A total of 41.5% of women had anemia, 46.3% had iron deficiency, and 55.0% had iron insufficiency. The prevalence of inflammation was also high: 27.9% had elevated CRP (> 5.0 mg/L) or AGP (> 1.0 g/L) levels, and 56.5% had CRP > 1.0 mg/L. After adjusting SF for inflammation, the prevalence of iron deficiency (61.5%) and iron insufficiency (72.7%) increased. The burden of iron deficiency, iron insufficiency, and IDA were significantly higher in WRA in rural areas compared to urban, although there were no differences in elevated CRP or AGP. The substantial burden of anemia and iron deficiency suggests an opportunity for anemia prevention.

4.1. Anemia

The prevalence of anemia (41.5%) and severe anemia (3.0%) in this study are consistent with recent WHO estimates on the burden of anemia in non-pregnant WRA (15-49 y), national data from India (57.2%; NFHS-5, 2019–2020) [63], and studies in WRA in other parts of India, where the prevalence of anemia ranged from 28% to 64% [64–67] and severe anemia ranged from 2.9% to 4.0% [65–67]. The prevalence of anemia in this study (41.5%) was slightly lower than the reported prevalence at the district (Chittoor: 51.8%) or state (Andhra Pradesh: 59.0%; urban: 57.8%, rural: 59.5%) level in the most recent NFHS-5 [68,69].. However, the use of capillary blood, inclusion of lactating women, and different time periods limit the comparability of findings [68,69].

4.2. Iron deficiency

This is the first study to date to replicate the recently described spline-based method for determining serum ferritin cutoffs for classifying iron deficiency in an international population-representative sample. Findings from these analyses were consistent with conventional cutoffs used in this setting. The high prevalence of iron deficiency (SF < 15.0 μg/L: 46.3%; SF < 12.0 μg/L: 40.6%) and iron insufficiency (SF < 20.0 μg/L: 55.0%) in this study is consistent with other studies among WRA in India (~25–64%): in a community based study in rural Telangana (SF < 12.0 μg/L: 55.7%; n = 979; not pregnant or lactating women, 15-35y) [64], and among adolescent females with anemia in New Delhi slums (SF < 15.0 μg/L; 41.1%; n = 794; 11-18 y) [70]. The prevalence of iron deficiency in this study was higher than in women in Hyderabad (SF < 15.0 μg/L: 36%; n = 156; not pregnant or lactating, >21 y) [71], female tea pickers in Darjeeling (SF < 12.0 μg/L: 25%; n = 212; not pregnant, 18-55 y) [72], and female adolescents in Himachal Pradesh (SF < 12.0 μg/L: 17.4%; n = 200, 11-19 y) [73] and Maharashtra (SF < 12.0 μg/L: 28%; n = 173; married, not pregnant or lactating, 15-19 y) [74]. In contrast, the prevalence of iron deficiency in this study was lower than in women in Bangalore (SF < 15.0 μg/L; 62%; n = 100; not pregnant or lactating, 18-35 y) [75] or in Haryana (SF < 15.0 μg/L; 63.8%; married, not pregnant; ≥18 y) [76].

In this study, the prevalence of iron deficiency defined by other biomarkers of iron status was high, including sTfR (> 8.3 mg/L:15.4%) and low total body iron (TBI < 0.0 mg/kg: 29.4%). Findings are similar to female tea pickers in Darjeeling (n = 212; not pregnant, 18-55 y; sTfR > 8.6 mg/L (Roche): 22%; TBI < 0.0 mg/kg: 21%) [72]. Reported sTfR levels were higher than observed in women in Bangalore (sTfR: GM: 2.01 mg/dL [95% CI: 1.7, 2.3]; n = 52; not pregnant or lactating, 18-35 y), although categorical sTfR indicators were not reported [77]. However, few studies to date have evaluated indicators of iron status in India apart from Hb or SF, and differences in laboratory methods (e.g., Ramco vs. Roche) and reported cut-offs constrain comparability of findings [78].

4.3. Inflammation

The prevalence of elevated inflammatory biomarkers was high in this study: 17.3% of WRA had CRP > 5.0 mg/L, 22.2% had AGP > 1.0 g/L, and 27.9% had either elevated CRP or AGP. Considering lower cut-offs for CRP which have been associated with cardiovascular risk, 29.9% of WRA had CRP > 3.0 mg/L and 56.5% had CRP > 1.0 mg/L. The reported CRP levels in this study (GM: 1.2 mg/L [95% CI: 1.1, 1.4]) were higher than previously observed in women in Bangalore (n = 52; not pregnant or lactating, 18-35 y; GM: 0.05 mg/dL [95% CI: 0.0, 0.86]) [77]. The prevalence of elevated CRP levels in this study was higher than in female tea pickers in Darjeeling (CRP > 5.0 mg/L: 5%; n = 212; not pregnant 18-55 y) [72] and in female students in New Delhi (CRP > 3.0 mg/L: 12.8%; n = 47; 14-25 y) [79]. Observed CRP levels were similar to those in female adolescents in Maharashtra (n = 401; 10-19 y; median: 1.26 mg/L; CRP ≥ 1 mg/L: 58.4%) [80], and lower than in women in urban slums in Mumbai (n = 200; 21–45 y; CRP ≥ 3 mg/L, 57%) [81], although cut-offs varied between studies. The prevalence of elevated AGP in this study was similar to female tea pickers in Darjeeling (AGP >1.0 g/L, 23%; n = 212; 18-55 y) [72]; however, there is limited population-based data on AGP in India.

In this study, after adjusting for inflammation, reported SF concentrations decreased as expected, and the prevalence of iron deficiency (unadjusted SF < 15.0 μg/L: 46.3 vs. BRINDA: 61.5% vs. Thurnham: 49.6%), iron insufficiency (unadjusted SF < 20.0 μg/L: 55.0 vs. BRINDA: 72.7% vs. Thurnham: 58.8%), and TBI < 0.0 mg/kg (unadjusted TBI < 0.0 mg/kg: 29.4% vs. BRINDA: 38.8% vs. Thurnham: 31.7%) all increased. In other studies that reported iron status in WRA in India, three collected CRP or AGP [71,72,75]; however, these studies did not adjust SF for inflammation [71,75], or did not report adjusted SF [72], which constrains comparability of findings.

4.4. Rural vs. urban

In this study, WRA in rural households had significantly higher prevalence of iron deficiency and iron insufficiency, compared to urban households, though the prevalence of anemia was similar. There were no differences in the prevalence of inflammation (CRP > 5.0 mg/L or AGP > 1.0 g/L), although CRP concentrations were significantly lower in rural areas. Findings were similar after adjusting SF concentrations for inflammation using BRINDA or Thurnham methods. Available data at the national level in India suggests the prevalence of anemia in WRA (15–49 y; rural: 58.58.65% vs. urban: 53.8%; NFHS-5, 2019-2020) [63] is similarly high in rural and urban settings. However, there is limited data on other iron biomarkers in India to which results can be compared.

4.5. Low consumption of animal source foods and iron status

In this study, the prevalence of iron deficiency and iron insufficiency were high, in combination with a low prevalence of self-reported vegan (0.3%) or vegetarian dietary patterns, as defined as lacto-vegetarian (3.6%, dairy), ovo-vegetarian (0.1%, eggs), lacto-ovo vegetarian (2.9%, eggs and dairy), pesco-vegetarian (0.3%, fish), or pollo-vegetarian (15.3%, poultry). However, of note is the low frequency of animal source foods (ASF) intake among non-vegetarian individuals (<5% reported meat or fish consumption >1/week). Dietary iron absorption varies by form: heme iron (exclusively in ASF) is readily absorbed, while absorption of non-heme iron (nuts, seeds, legumes) varies and its bioavailability is influenced by other food components (e.g., phytates, tannins). The low frequency of ASF intake, even in non-vegetarian individuals in this population is consistent with previous studies in India. For example, a study among pregnant women in Uttar Pradesh [82] found that daily iron intake did not meet the recommended dietary allowance or differ by self-reported diet preference (i.e., lacto-vegetarian vs. non-vegetarian).

The World Health Organization recommendations for iron assessment of populations include Hb, SF, sTfR, and at least one acute phase protein, such as CRP or AGP [83]. However, few national or population-representative surveys collect data on iron biomarkers beyond Hb, and many surveys evaluate Hb as a proxy for iron deficiency [21,37]. This study is population-based, among the largest of its kind to date in India, and included a comprehensive assessment of iron status, including Hb, SF, sTfR, CRP, and AGP, per WHO recommendations. sTfR is a carrier protein required for iron endocytosis that is regulated in response to intracellular iron levels and is less sensitive to inflammation or the acute phase response in infection. Measurement of both SF and sTfR, and use of different methods to adjust for inflammation, allows for differentiation of the effects of inflammation on host iron status and changes in iron status.

This study has several limitations. Due to the cross-sectional study design, it is not possible to evaluate changes in biomarkers over time or to establish temporal or causal relationships. Additionally, this study was not designed to evaluate risk factors (e.g., socioeconomic, dietary) for iron deficiency. Although this study included WRA who were not pregnant or lactating, it was not designed to evaluate the micronutrient status of WRA who were intending to become pregnant or the pre-conception period. The age range for WRA in this study (15–40 y), constrains comparability to some studies in the literature with different definitions of WRA and inclusion of lactating women. The response rate was ~54%; although participants in the biomarker study were similar to the periconceptional surveillance program, they may differ on other unmeasured factors. Inflammation is associated with a variety of human disorders and is common in the context of obesity, undernutrition, and infectious diseases. The lack of assessment of hepcidin or inflammatory cytokines that influence iron status is a limitation that might be addressed in future studies.

5. Conclusion

In this population-based biomarker survey, there was a substantial burden of anemia, iron deficiency, and inflammation. The estimated prevalence of iron deficiency and insufficiency increased further after adjusting for inflammation in this population, suggesting a potential to benefit from comprehensive screening for biomarkers of inflammation as part of interventions to improve iron status. Further research may help elucidate the complex etiology of anemia and iron deficiency in WRA and links to health outcomes and inform decisions on screening and interventions to improve the health of women and young children. The substantial burden of anemia and iron deficiency in WRA indicates an opportunity for prevention. Findings provide pre-intervention iron biomarker data for a randomized trial for anemia prevention in this population.

Author contributions

JLF designed the research and wrote the initial draft manuscript, with feedback from the study collaborators; AF, HMG, RK, and KSC revised the manuscript. WB, CBJ, and AF supervised data collection and field activities. BB conducted the laboratory analyses. AF conducted data analyses. All authors contributed to the development of the manuscript, provided feedback, and read and approved the final version. JLF had primary responsibility for the final content.

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Declaration of competing interest

The authors have no conflicts of interest to disclose related to this manuscript. SM is an unpaid board member and holds equity in a startup commercializing point-of-care diagnostic technology partially developed in his research laboratory at Cornell University. Findings and conclusions in this report are those of the authors and do not necessarily represent the official positions of the Centers for Disease Control and Prevention, the DRDC, Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), or the National Institutes of Health.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnesp.2022.02.123.

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