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Low iron stores in preconception nulliparous women; a two-center cross-sectional study in peri-urban Ghana

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ABSTRACT

Objectives: The nutritional status of women affects pregnancy outcomes. Preconception care adequately prepares women for the demands of pregnancy. The aim of this study was to evaluate the prepregnancy iron status of nulliparous women in Ghana to provide empirical data to inform policy formulation.
Methods: We recruited 336 nulliparous women of reproductive age from Denkyembour District and Ejura Municipal assemblies, respectively, in the Eastern and Ashanti regions of Ghana. Serum ferritin was estimated to assess the women's iron stores. Hemoglobin (Hb) variants and fecal occult blood were determined for participants using cellulose acetate electrophoresis and lateral flow chromatographic immunoassay, respectively. Logistic regression analysis was used to determine factors associated with depleted iron stores (ferritin $<$ 15 ng/dL).
Results: Whereas 41.5% of the women were anemic (Hb $<$11.5 g/dL), 34.5% were iron depleted (serum ferritin $<$15 g/mL; C-reactive protein $<$5 ng/dL). Also, 17.1% suffered from iron deficiency anemia (concomitant Hb $<$11.5 g/dL and serum ferritin $<$15 ng/dL). Serum ferritin significantly differed among the participants with anemia (P $<$ 0.001). Multivariate regression analysis showed that age ($<$20 y: adjusted odds ratio [AOR], 13.916, P = 0.002; 20–30 y: AOR, 4.304, P = 0.023), moderate anemia (AOR, 3.045, P = 0.004), Ashanti region (AOR, 3.984, P = 0.002), and mean cell volume $<$80 fL (AOR, 2.546, P = 0.003) were significantly associated with increased odds of having depleted iron stores. However, body mass index, waist-to-hip ratio $>$0.85, educational status, or inherited Hb type were not significantly associated with depleted iron stores.
Conclusion: The high prevalence of depleted iron stores in nulliparous women is a severe public health problem that requires attention.

Introduction

Preconception care is a concept that has been proposed to address maternal health problems and environmental risk factors in the prepregnancy period so as to improve gestational outcomes [1]. Practically, preconception care strategies translate as behavioral, biomedical, and social interventions offered to couples before conception occurs so that some preventable diseases could be avoided with improved lifestyle choices. In developing countries, any such strategies must include anemia in the broadest sense, and particularly iron deficiency anemia (IDA). These two conditions are important considerations owing to their high prevalence in these environments and their consequent adverse health effects, such as increased maternal and infant mortality, retarded mental and physical development, and impaired cognitive function [2]. As reported elsewhere, anemia occurs in ~500 million women of reproductive age in resource-poor countries [3]. For example, anemia prevalence was 62% among pregnant women in Vietnam despite iron supplementation being recommended for all pregnant women [4]. A study conducted in Ghana indicated that the prevalence of anemia among children under 5 y of age and in women between 15 and 49 y of age were 78.4% and 42%, respectively [5,6].

PA and RKDE conceived the study and participated in the design. YAA, PA, AW, and STN were involved in data acquisition, laboratory work, and literature search. PA analyzed the data and drafted the manuscript. RKDE and YAA critically reviewed the manuscript. All authors read and approved the final manuscript. The authors have no conflicts of interest to declare.

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In light of similar distressing statistics in other developing countries, the World Health Organization (WHO) has recommended iron supplementation to improve iron stores in adolescent girls and women during the preconception period, particularly in areas where anemia prevalence is >40% [7]. In cases where mass nutritional fortification cannot be attained, and where the prevalence of anemia in women of reproductive age is >20%, the WHO recently issued a position statement recommending weekly iron–folic acid supplementation [8].

This preconception iron supplementation is meant to meet the ~1000 mg of iron required during pregnancy [9]. It has been stated that the incidence of iron deficiency in pregnancy is underscored by two factors: prepregnancy iron stores and the amount of iron absorbed during pregnancy [10]. Although there is increased iron absorption during pregnancy [11], this increased absorption is generally unable to compensate for inadequate prepregnancy stores, leading to higher prevalence of IDA, such as 33% in Zimbabwe [12] and as high as 72% in the aboriginal population >14 y of age in North-West Australia [13].

In Ghana, most of the interventions offered to women are targeted mostly during the post-pregnancy period. Research, however, has demonstrated that interventions offered during pregnancy do not adequately cater to the nutritional needs of mother and fetus [14,15]. Given that IDA is prevalent in the general Ghanaian population, it is not far-fetched to suppose that most women conceive while already in an iron-deficient state. However, preconception care targeting nulliparous women has not received much attention as a public health issue probably because of a scarcity of data. In order to rule out the confounding influence of previous pregnancy on iron stores, we limited the scope of the present research to nulliparous women. We sought to evaluate the prepregnancy iron status of nulliparous women in Ghana to provide empirical data that would stimulate discussion and ultimately inform policy formulation.

Methods

Study design and study site

A simple convenience sampling of nulliparous women was conducted to ascertain iron status. The study participants comprised nulliparous women in the reproductive age group (16–36 y) residing in and around Denkyembour district in the Eastern region of Ghana and the Ejura district in the Ashanti region of Ghana. The two sites were selected to reflect the zonal division of Ghana: Denkyembour District (southern zone) and Ejura Municipal (northern zone). Additionally, the anemia prevalence of the two regions mirrors the national average [16]. We recruited 336 participants (who had had their menstrual flow within the previous 2 wk) with 186 from Denkyembour district and 150 from Ejura district. The study initially consecutively recruited 200 participants from each of the study sites; however, samples from participants who were not able to provide stool samples, or those who refused to disclose their menstrual status were removed from the analysis. Also, respondents with acute liver diseases or any inflammatory conditions were excluded as these conditions significantly affect ferritin levels. Also, participants were ≥3 d post last menstrual flow, but within 2 wk of last menstrual flow.

Questionnaire

A well-structured, closed-ended questionnaire was administered to each participant to help obtain information on sociodemographic variables such as age, region, occupation, educational status, marital status, and iron supplementation.

Anthropometrics

Height (to the nearest 0.1 m) was measured using a wall-mounted graduated ruler. Weight (to the nearest 0.1 kg), with participants wearing lightweight clothing, was also measured with a weighing balance (Seca, Hamburg, Deutschland). Body mass index (BMI) was calculated as the ratio of the weight (kg) and the square of the height (m^2). BMI was categorized as underweight (<18.5 kg/m^2); normal weight (18.5–24.5 kg/m^2); overweight (25–29.5 kg/m^2); and obese (≥30 kg/m^2). Waist circumference (WC) was measured from the midpoint between the lower margin of the palpable rib and top of the iliac crest using a stretch-resistant tape in accordance with WHO recommendations [17]. Hip circumference (HC) was also measured around the widest portion of the buttocks. WC (cm) and HC were then used to estimate the waist-to-hip ratio (WHR).

Laboratory assays

Five mL of blood was taken from the forearm of each participants; 3 mL into ethylenediamine tetracetic acid (EDTA) tube and 2 mL into serum separator tube (SST) for the laboratory assays, as explained later. All sampling was undertaken between 0800 h and 1000 h after an overnight fast. The EDTA anticoagulated samples were used for complete blood count (CBC), hemoglobin (HB) electrophoresis, and malaria parasite count. Samples in the SST were allowed to clot, spun at 5000*g* for 5 min to obtain serum, transferred into Eppendorf tubes and stored at −25°C until required (for serum ferritin and C-reactive protein [CRP] estimation). Each participant provided a stool sample for occult blood estimation.

Complete blood count

Participants' Hb levels were estimated with the Sysmex (XS 500 i) hematology analyzer (Sysmex Corporation, Kobe, Japan) in accordance with manufacturer's specifications. Based on WHO guidelines [7], anemia was defined as Hb <11.5 g/dL.

Hb electrophoresis

The Hb types of participants were determined using cellulose acetate electrophoresis (pH 8.2–8.4) in accordance with previously published protocols [18].

C-reactive protein

Serum CRP was estimated using the enzyme-linked immunosorbent assay (ELISA) method in accordance with manufacturer's specifications (R&D Systems China Co., Ltd., China). Plates were then read on the URIT -660 Microplate Reader (URIT Medical Electronic Co., Ltd., Guangxi, P.R China). As per WHO recommendation, CRP cutoff for no inflammation was taken as ≤5 mg/L [19]. Of the 336 participants recruited, 20 (5.5%) had CRP >5 mg/L and were excluded from further analyses.

Serum ferritin

Serum ferritin was estimated using the ELISA method in accordance with manufacturer's specifications (Chemux Bioscience Inc., South San Francisco, CA, USA). All plates were read on the URIT-660 Microplate Reader (URIT Medical Electronic Co.) Low iron store was defined as ferritin levels <15 ng/mL in the absence of inflammation [19,20].

Malaria parasite density estimation

Malaria parasite density was estimated with the Giemsa stained thick blood film technique described elsewhere [21].

Fecal occult blood test

Fecal occult blood test (FOBT) was done using the fecal occult blood One-Step diagnostic test kit (Clinogen Diagnostics, Berkshire, UK). Participants were properly instructed on the best way to collect fecal samples. Women in the menstrual stage of their cycle were excluded until they were ≥3 d post-menstruation. Participants were also advised to avoid intake of vitamin C, aspirin, or non-steroidal anti-inflammatory drugs for ≥7 d before fecal sampling. Additionally, participants were instructed not to consume red meat products the day before the sampling so as not to confound FOBT results.

Ethical consideration

All protocols for the study were approved by the Institutional Review Board, University of Cape Coast. No personal identifiers were collected or used during data collection. All participants gave written informed consent before being recruited into the study.

Statistical analysis

All analyses were performed using SPSS version 20 (IBM, Chicago, IL, USA). Continuous variables were expressed as mean ± SD. To analyze the differences between groups, the independent *t* test was used for continuous variables, and χ^2 test was used for nominal variables. Factors associated with depleted iron stores (ferritin levels <15 ng/mL) were predicted using logistic regression analysis; age, education, region of residence, occupation, Hb, mean cell volume (MCV), BMI, WHR, FOBT, Hb variants, and iron supplementation were entered as the independent categorical variables in the regression models. $P < 0.05$ was considered statistically significant.

Table 1
Socidemographic parameters of participants with normal C-reactive protein values

Parameter	n (%)	*P*-value
Age, y		<0.001
<20	37 (11.7)	
20–29	252 (79.7)	
≥30	26 (8.3)	
Marital status		<0.001
Single	279 (88.3)	
Married	37 (11.7)	
Education		<0.001
Basic	60 (19)	
Secondary	49 (15.5)	
Tertiary	207 (65.5)	
Occupation		<0.001
Teacher	20 (6.30)	
Nurse	101 (32)	
Student	72 (22.8)	
Self-employed	48 (15.2)	
Civil servant	33 (10.4)	
Unemployed	42 (13.3)	
Region		0.033
Ashanti	139 (44)	
Eastern	177 (56)	
Iron supplementation		<0.001
No	255 (80.7)	
Yes	61 (19.3)	

Results

Table 1 stratifies the demographic characteristics of the participants who had a normal CRP value. A significantly higher proportion of the nulliparous women were 20 to 29 y of age ($P < 0.001$). Also, a significantly higher proportion of the participants were single ($P < 0.001$; 88.3% single versus 11.7% married). All participants had attained some level of education, with a significant proportion having tertiary education. Furthermore, a significantly lower proportion of the participants were taking iron supplementation ($P <$ 0.001, 19.3% taking iron supplementation versus 80.7% not taking iron supplementation).

Of the 316 participants with normal CRP values, 41.5% had anemia, with 1.6% being severely anemic (Table 2). Additionally, 34.5% had depleted iron stores, as shown by ferritin levels <15 ng/mL. Also, fewer participants demonstrated blood loss through the gastrointestinal tract ($P < 0.001$; 94.3% negative for fecal occult blood versus 5.7% positive for fecal occult blood). Moreover, whereas 13.6% of the participants demonstrated Hb variants as measured by electrophoresis, only 1.9% had asymptomatic malaria. Furthermore, whereas 31.3% of the participants had abnormal BMI, only 6.3% demonstrated abnormal WHR (>0.85).

Furthermore, 17.1% (54) of the 316 study participants had IDA as defined by concomitant ferritin levels of <15 ng/mL and Hb levels <11.5 g/dL (Table 3) [20]. Also, ferritin levels significantly differed across the anemia subcategories ($P < 0.001$; $\chi^2 = 20.251$). However, ferritin levels did not significantly differ across the age categories ($P < 0.102$; $\chi^2 = 4.588$).

Multivariate logistic regression analysis was used to assess factors associated with depleted ferritin levels (ferritin levels <15 ng/mL) among the participants (Table 4). The regression analysis showed that age (<20 y: adjusted odds ratio [AOR], 13.916, $P = 0.002$; 20–30 y: AOR, 4.304, $P = 0.023$), moderate anemia (AOR, 3.045, $P = 0.004$), Ashanti region (AOR. 3.984, $P = 0.002$), and MCV <80 fL (AOR, 2.546, $P = 0.003$) were significantly associated with increased odds of participants having depleted ferritin or iron stores. Additionally, although deranged BMI, severe anemia, positive FOBT, or not taking iron supplementation were associated with increased odds of having depleted iron stores, none of these reached statistical significance.

Table 2
Anthropometric and hematobiochemical characteristics of participants without inflammation

Parameter	n (%)	*P*-value
Anemia classification		<0.001
Severe: Hb <7 g/dL	5 (1.6)	
Moderate: Hb 7–10 g/dL	50 (15.8)	
Mild: Hb >10–<11.5 g/dL	76 (24.1)	
No anemia	185 (58.5)	
Ferritin levels, ng/mL		<0.001
<15	109 (34.5)	
≥15	207 (65.5	
Fecal occult blood		<0.001
Negative	298 (94.3)	
Positive	18 (5.7)	
Malaria parasites		<0.001
Negative	310 (98.1)	
Positive	6 (1.9)	
Hb variants		
AS	17 (5.4)	
AC	26 (8.2)	
A	273 (86.4)	
BMI, kg/m^2		<0.001
Underweight: <18.	6 (1.9)	
Normal: 18.5–24.5	217 (68.7)	
Overweight: 25–29.9	75 (23.7)	
Obese: ≥30	18 (5.7)	
WHR		<0.001
<0.85	296 (93.7)	
≥0.85	20 (6.3)	

A, only haemoglobin A band was detected at alkaline pH; AC, haemoglobin A and C bands were detected; AS, haemoglobins A and S bands were detected; BMI, body mass index; Hb, hemoglobin; WHR, waist to hip ration

Discussion

Preconception care is increasingly gaining attention as the most effective means to prevent gestational, peripartum, and postpartum maternal and fetal complications. This study sought to estimate the status of iron stores among nulliparous women from two selected districts in Ghana. The findings showed 41.5% of the participants were anemic as per 11.5 g/dL Hb level cutoff [7], which is a severe public health problem [22]. This is suggestive of the need to consider policies that include iron supplementation for nulliparous women within the study population as the prevalence reported herein falls under the severe public health problem category recommended by the WHO [7]. Previous studies have,

Table 3
Hemoglobin and ferritin level cross-tabulation

Parameter	Ferritin level (ng/mL)		χ^2 value	*P*-value
	<15	>15		
Hemoglobin (g/dL)				
Severe anemia	4_a	1_b	20.251	**<0.001**
Moderate anemia	29_a	21_b		
Mild anemia	21_a	55_a		
No anemia	55_a	130_b		
Total	109	207		
Age, y				
<20	14_a	23_a	4.588	0.102
20–30	90_a	162_a		
>30	4_a	22_b		
Total	108	207		

Subscript letters denote a subset of ferritin level (ng/mL) categories whose column proportions do not differ significantly from each other at the 0.05 level.
p-values in bold print indicates statistical significance ($p < 0.05$).

Table 4
Factors associated with depleted iron stores (ferritin levels <15 ng/mL)

Parameter	*P*-value	AOR	95% CI
Age, y			
<20	**0.002**	13.916	2.55–75.949
20–30	**0.023**	4.304	1.219–15.194
>30	Reference		
Marital status			
Single	0.994	0.996	0.391–2.537
Married	Reference		
Education			
Primary	0.056	0.372	0.135–1.024
Secondary	0.215	0.576	0.241–1.378
Tertiary	Reference		
BMI, kg/m^2			
<18.5	0.837	1.233	0.168–9.036
18.5–24.5	Reference		
25–29.9	0.850	1.059	0.581–1.932
>29	0.968	1.025	0.310–3.286
WHR			
<0.85	Reference		
≥0.85	0.426	0.632	0.205–1.953
Anemia category, g/dL			
Severe: Hb <7	0.099	50.116	0.480–5234.112
Moderate: Hb 7–10	**0.004**	3.045	1.421–6.524
Mild: Hb >10–<11.5	0.827	0.928	0.475–1.812
No anemia: Hb ≥11.5	Reference		
FOBT			
Negative	Reference		
Positive	0.122	2.741	0.764–9.838
Malaria parasite			
Present	0.312	0.231	0.130–3.952
Nil	Reference		
Hb electrophoresis			
AS	0.726	0.810	0.249–2.630
AC	0.516	0.701	0.240–2.049
A	Reference		
Region			
AR	**0.002**	3.984	1.641–9.675
ER	Reference		
Iron supplementation			
Yes	Reference		
No	0.332	1.409	0.705–2.818
MCV, fL			
<80	**0.003**	2.546	1.388–4.670
80–98	Reference		
>98	0.943	0.969	0.406–2.310

AOR, adjusted odds ratio; BMI, body mass index; FOBT, fecal occult blood test; MCV, mean cell volume; WHR, waist-hip ratio.
p-values in bold print indicates statistical significance ($p < 0.05$).

however, reported lower cases of anemia among this population in different research locations: 15.38% [23] in Mexico and 12% [24] in Nepal. The differences could be attributed to the variations in the geographic locale of the participants in the various studies. For example, although Chandyo et al. reported 12% anemia prevalence in Nepal [24], other studies in other parts of Nepal have reported anemia prevalence of 60% [25,26].

We also identified that 34.5% of the present participants had depleted iron stores (i.e., ferritin levels <15 ng/mL). This is not surprising considering that the majority of our participants relied on traditional non-fortified diet to cater to their nutritional needs. Previous studies that used serum ferritin as the sole indicator of body iron stores have given 20% (in Nepal), 23.10% (Mali), and 25% (Vietnam) prevalence of iron deficiency among women of reproductive age [24,27,28]. However, another cross-sectional study among women of reproductive age in Cambodia reported 8.1% prevalence of low iron stores [29]. The differences may be attributed to the variations in geographic locations and nutritional habits and the different cutoff values for serum ferritin used. IDA, defined by concurrent Hb <11.5 g/dL and serum ferritin <15 ng/mL, was 17.1% in the present study. IDA has been given for this population in different settings as 39% (cross-sectional study in Kazakhstan) [30], and 15.5% (cross-sectional study in Mexico) [31]. Interestingly, iron supplementation among the participants was only 19.3%, despite WHO recommendations of iron supplementation for women of reproductive age among populations where anemia prevalence is >20% [8]. Our findings of 41.5% anemia prevalence, 34.5% iron depletion prevalence, and 17.1% prevalence of IDA, is a grave public health concern considering that these are reproductive-age women who could conceive at any given time and, without adequate preconception iron stores, may not be able to meet the ~1000 mg iron required per normal pregnancy [9]. We believe that these adverse findings necessitate the adoption of routine iron supplementation policy in nulliparous women in the study population, as this has been recommended as a means of helping build iron stores among adolescent girls and women of reproductive age [32]. However, there should not be *en bloc* supplementation, but that those with iron deficiency states should be fully investigated and underlying causes treated.

This study further demonstrated that the odds of having depleted iron stores in nulliparous women are significantly higher in teenagers, those <30 y of age, those with moderate anemia, and those with MCV <80 fL. It is well documented that the increased blood volume, increased Hb mass, monthly blood loss, and increased physical and cognitive development associated with adolescence all require adequate supplies of iron [33–35]. Such increased developmental requirements may explain the higher odds found in the adolescents within the present study cohort. The consequences of depleted iron stores on adolescent health in the present study population as well it its economic burden, in the event of pregnancy, remains to be quantified in future well-controlled longitudinal studies. Because iron deficiency is one of the common causes of microcytic anemia [36], it is not surprising that MCV <80 fL (microcytosis) was associated with higher odds of having depleted iron stores. Although the reason(s) for the increased odds of participants from the Ashanti region having depleted iron stores was not readily apparent, it is interesting to note that a higher percentage of the participants from the Eastern region had tertiary education compared with those from the Ashanti region (82.2% versus 44%). We speculate that these differences in educational backgrounds and other environmental-related factors may be responsible for the variations in iron status among participants from the two regions.

In agreement with a previous cross-sectional study in Mali [27] that recruited reproductive-aged women, we did not find a significant association between serum ferritin and weight status. Similarly, another cross-sectional study in the United States [37] that investigated iron status in premenopausal women did not find any significant association between BMI or body adiposity and iron status. However, this finding contrasts with other studies that found overweight to be inversely associated with iron status [38–43]. This may be a function of the differences in the population recruited, geographic location, or methods employed in the various research. For example, studies by Pinhas-Hamiel et al. [41] and Nead et al. [43] recruited adolescents and children, whereas adolescents comprised only 11.7% of the participants in the present study. Also a study by Lecube et al. recruited postmenopausal women [42], whereas this study recruited women in their reproductive age.

Although this study revealed interesting public health challenges that must be addressed, there were some obvious limitations. Chief among these was our inability to evaluate the participants for intestinal parasitic infections. Although FOBT provided a means of eliminating the confounding effects of most of these intestinal parasites, addition of such data could have enriched the scope of our findings. Additionally, measurement of soluble transferrin receptor for the body iron stores determination would have increased the robustness of our analyses. Moreover, we were unable to collect data on frequency, duration, and volume of menstrual flow, which may have given further insight into the causes of anemia and low iron stores. Future studies should address these as well as collect food diaries to assess dietary intake of iron-containing foods.

Conclusion

This study found the prevalence of anemia, iron-depleted state, and IDA to be 41.5%, 34.5%, and 17.1%, respectively. There is a need to establish the etiologies of this severe public health problem and measures should be put in place to improve future pregnancy and other health-related outcomes.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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