

Maternal Anemia at First Antenatal Visit: Prevalence and Risk Factors in a Malaria-Endemic Area in Benin

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Abstract. The risk factors for maternal anemia (hemoglobin level less than 110 g/L) were studied in human immunodeficiency virus–negative pregnant women in Benin at the time of first antenatal visit and prior to any prevention. Data for the first 1,005 pregnant women included in a multicentre randomized controlled trial were analyzed. Anemia was common (68.3%), and malaria and helminth infestations were prevalent in 15.2% and 11.1% of the women. A total of 33.3%, 31.3% and 3.6% of the women were iron, folic acid and vitamin B12 deficient, respectively. These parasitic infections and nutrient deficiencies were associated with a high risk of anemia. Twenty-one percent, 15%, 12%, 11% and 7% of anemia were attributable to malnutrition, malaria, iron, folic acid deficiencies, and helminth infestations, respectively. Most anemia was caused by factors that could be prevented by available tools, stressing the need to reinforce their implementation and to evaluate their effectiveness throughout the course of the pregnancy.

INTRODUCTION

Anemia in pregnancy, defined as a hemoglobin concentration less than 110 g/L, remains one of the greatest public health concerns in developing countries.¹ It is extremely common and prevalence rates ranging from 35% to 75% have been reported.² Severe anemia (hemoglobin level less than 70 g/L) is present in 5–10% of the cases, and induces the most dramatic consequences, i.e., increased risk of maternal morbidity and mortality, abortion, poor intrauterine growth, preterm birth and low birth weight.^{3,4} These effects in turn result in higher perinatal morbidity and mortality, and higher infant mortality rate.⁵

Although the pathogenesis of anemia is multifactorial,^{6,7} the disease is thought to be mainly caused by iron deficiency (ID) in developing countries and therefore, iron supplementation is routinely recommended⁸ as the main prevention measure against anemia. In sub-Saharan Africa where ID is common, the prevalence of anemia has often been used as a proxy for iron deficiency anemia (IDA),⁹ although no study has so far definitely established a significant relationship between iron status and anemia in pregnant women.^{10,11}

Infectious and parasitic diseases, in particular malaria, helminth infestations and urinary tract infections are also important factors contributing to the high prevalence of anemia in sub-Saharan Africa.^{7,11,12} Helminth infestations, especially hookworm and schistosomiasis, cause blood loss and thus contribute to increase the risk for anemia in pregnancy. The role of other factors, such as folic acid and vitamin B12 deficiencies or hemoglobinopathies also needs to be assessed precisely, in particular to determine the preventable causes of anemia.

On the occasion of a multi-center trial of Intermittent Preventive Treatment in pregnancy (IPTp) comparing sulfadoxine-pyrimethamine and mefloquine (MiPPAD study “Malaria in Pregnancy Preventive Alternative Drugs”, [\[.gov/ct2/show/NCT00811421\]\(http://clinicaltrials.gov/ct2/show/NCT00811421\)\) funded by the European and Developing Countries Clinical Trials Partnership \(EDCPT\), we had the opportunity to follow-up the first 1,005 women included at the study site in Benin to investigate the prevalence and the risk factors of maternal anemia throughout pregnancy \(study “Anemia in Pregnancy: Etiologies and Consequences”\). We present the results of our investigations at the time of the first antenatal visit \(ANV\) before any supplementation or antihelminthic treatment.](http://clinicaltrials</p></div><div data-bbox=)

MATERIALS AND METHODS

Study design. The study was a cross-sectional survey conducted at the inclusion of the first 1,005 pregnant women participating in the MiPPAD trial.

Study site. The study was conducted in the district of Allada, a semi rural area located 50 km north of Cotonou, the economic capital of Benin. The entire district is made of 12 sub-districts, 84 villages, and a total of 91,778 inhabitants. The study participants were recruited in three maternity clinics in three sub-districts: Allada, Attogon, and Sékou. There are several ethnicities living in the district of Allada, the most important being Aïzo, a local ethnic group. Malaria is perennial and *Plasmodium falciparum* is the most common species. There are two high transmission peaks from April through July and October through November. Transmission is low during the rest of the year.

Study population. The study population was composed of human immunodeficiency virus (HIV)–negative pregnant women (less than 28 weeks of gestational age) residing in the district of Allada, who attended the ANV at any of the three maternity clinics for the first time during January 2010–May 2011. The eligibility criteria included no intake of IPTp, iron, folic acid, vitamin B12, or anti-helminthic treatment, which are part of the ANV package in Benin, since the beginning of the pregnancy. All women were offered confidential pre-test HIV counseling and thereafter informed consent was obtained for blood sample collection.

Study procedures. *Sociodemographic and clinical data collection.* All pregnant women who attended any of the three maternity clinics for ANV were approached to participate in

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the study. After informed consent was obtained, they were screened for inclusion and exclusion criteria and socio-demographic data such as age, parity, area of residence, marital status, level of education, occupation, and information useful to determine the socioeconomic level were recorded. They were clinically examined and gestational age (assessed by measuring the fundal height), mid upper-arm circumference, weight, and height were evaluated. Weights were measured to the nearest 0.1 kg by using an electronic scale (to ± 100 grams; Seca Corp., Hanover, MD) and heights to the nearest 0.1 cm by using a bodymeter device (Seca® 206 Bodymeter; Seca Corp.). Weights and heights were measured by two nurses, and the mean of the two measurements was calculated for each participant. Information on previous pregnancies and children and history of chronic diseases were also recorded.

Blood and fecal samples collection at enrollment. Eight milliliters of venous blood was obtained from each participant, of which 4 mL was collected into a tube containing dipotassium EDTA and 4 mL was collected into an iron-free dry tube. Blood samples were collected before the administration of hematinics, folic acid, IPTp, or antihelminthic drugs as part of antenatal prophylaxis.

Containers were given to the women to collect feces for intestinal helminth infestations. These containers were collected the next day within the first six hours after defecation.

Laboratory tests. The hemoglobin level was measured with a Hemo-Control photometer (EKF Diagnostics, Barleben/Magdeburg, Germany) device with 10 μ L of blood. Daily calibration of the Hemo-Control device was performed by laboratory technicians. In addition, an external quality control was made by sending one of 10 consecutive samples to the Allada Central Hospital laboratory, where dosages were determined by using a hematology analyzer (Erma Laboratory, Tokyo, Japan).

Women with a hemoglobin concentration less than 110 g/L were treated according to the national guidelines, i.e., 200 mg of iron twice a day for mild and moderate anemia (hemoglobin levels = 70 g/L and less than 110 g/L, respectively), and treated locally or referred to the tertiary hospital of the district in case of severe anemia (hemoglobin level less than 70 g/L). Furthermore, women were advised to consume iron-rich foods, such as beef, eggs, and green leafy vegetables. Hemoglobin genotypes were determined by using alkaline electrophoresis on cellulose acetate (Helena Laboratories, Beaumont, TX) with 50 μ L of blood.

Serum ferritin, folic acid, and vitamin B12 concentrations were measured by using an AxSym Immuno-Assay Analyzer (Abbott Laboratories, Abbott Park, IL) with 500 μ L of serum. C-reactive protein (CRP) concentrations were determined by using a rapid slide test (CRP Latex; Cypress Diagnostics Inc., Campbellville, Ontario, Canada) to correct for increased ferritin levels associated with inflammatory syndromes.¹³

Detection of HIV detection is part of the first ANV package in Benin. The Determine (HIV 1 and 2 Kit; Abbott Laboratories) and Bioline (HIV 1 and 2 3.0 Kit; Bioline, Taunton, MA) rapid tests were used to detect HIV infections by using a serial testing algorithm. HIV-positive tests were sent to the District of Allada Central Hospital for confirmation by using an enzyme-linked immunosorbent assay. When an HIV-positive result was confirmed, she was treated according to the Benin National Program against HIV and

Acquired Immunodeficiency Syndrome Guidelines (Program National de Lutte Contre le VIH/SIDA).

The Lambaréné technique was used to assess malarial infection. Ten microliters of blood was spread on a rectangular area of 1.8 cm² (1.8 cm \times 1 cm) of a slide. The slide was stained with Giemsa and read at a magnification of 1,000 \times with an oil immersion lens. A multiplication factor was applied to the average parasitemia/field to determine the number of parasites/microliter. The Lambaréné method detection threshold has been estimated to be 5 parasites/ μ L.¹⁴

Infestations by helminths were assessed by using the Kato-Katz concentration method¹⁵ (Vestergaard Frandsen, Lausanne, Switzerland). Because fecal samples must be processed and examined extemporaneously, no external control was used, but the slides were read by two laboratory technicians independently.

Definitions. Anemia. For the definition of severe anemia, we preferred to use a more discriminating 80 g/L threshold than the 70 g/L recommended by the Beninese Ministry of Health. Anemia was defined as a hemoglobin concentration less than 110 g/L. Severe, moderate, and mild anemia were defined as hemoglobin concentrations less than 80 g/L, 80–99 g/L, and 100–109 g/L, respectively.

Iron status and IDA. Iron deficiency was defined as a serum ferritin concentration less than 12 μ g/L or as serum ferritin concentration of 12–70 μ g/L in the context of inflammatory syndrome.¹⁶ Iron deficiency anemia was defined a hemoglobin concentration less than 110 g/L with ID. Inflammation was defined as positive CRP result, i.e., CRP concentration less than 5 mg/mL.¹⁷

Folic acid and vitamin B12 deficiencies. Folic acid deficiency was defined as a serum folic acid concentration less than 6 ng/mL. Vitamin B12 deficiency was defined as a vitamin B12 serum concentration less than 150 pg/mL.

Helminth infestations. Intestinal helminth infestations were diagnosed by the presence of intestinal helminth eggs in the fecal sample. Eggs were counted (number of eggs/gram of feces).

Body mass index at the beginning of pregnancy. From the end of the first trimester of gestation, pregnant women gain 1 kg per month until delivery.¹⁸ We used the gestational age at inclusion to estimate the weight (in kilograms) that women were supposed to gain since the beginning of the pregnancy. This amount was then subtracted from the weight on the day of inclusion to obtain a rough estimate of the weight before pregnancy (Table 1).

Data management and statistical analysis. Data were collected in parallel to the MiPPAD study by using the source documents from the trial. They were double-entered into an Microsoft (Redmond, WA) Access database and analyzed by using with Stata Software for Windows version 11.0

TABLE 1
Estimation of body mass index at beginning of pregnancy in Benin*

Gestational age (weeks)	Weight at beginning of pregnancy (kg)
8–12	W1
13–16	W2
17–20	W3
21–24	W4
25–28	W5

* Body mass index (kg/m²) = W/H \times H, where W = weight at inclusion in kilograms and H = height in meters.

(StataCorp LP, College Station, TX). We first described the baseline characteristics of the women and the factors potentially influencing their hemoglobin levels. Means of hemoglobin concentrations were computed and compared by using the Students *t*-test.

Relationships between anemia and risk factors were studied by using univariate logistic regression. A multiple logistic regression was performed that took into account all factors with *P* values < 0.20 by univariate analysis.

Population-attributable risks were also calculated to estimate the proportion of anemia that could be prevented by the elimination of each of the assessed risk factors. A *P* value below 0.05 was considered statistically significant.

Ethical considerations. This study was approved by the Ethics Committee of the Faculty of Medicine of Cotonou, Bénin. Before each inclusion, the study was explained in local language to the participant and her voluntary consent was obtained. In case the woman could not read, an impartial witness was involved in the process. In addition to the assent of minors, consent was obtained from the parents or legal guardians. Women were free to interrupt their participation at any time during the study.

RESULTS

In this study, 1,008 pregnant women were enrolled. Three women were excluded from the analysis because of HIV-positive results, uterus fibroma, and gestational age more than 28 weeks, respectively. Among the 1,005 remaining pregnant women, helminth infestations and hemoglobin genotypes were not assessed in 16 participants and 1 participant, respectively. All remaining data were obtained from the entire study population.

General characteristics of the study population. Only 8% of the women were consulted at the health center for the first time during the first trimester (< 16 weeks of gestation), and the remainder (92%) were consulted during the second trimester. Women had a mean age of 25.8 years (95% confidence interval [CI] = 5.4–6.1 years and 18.9% were primigravidae. The mean gestational age at the first ANV was 22.1 weeks (95% CI = 21.8–22.3 weeks). The mean hemoglobin level was 103.2 g/L (95% CI = 102.4–103.9 g/L), and the mean body mass index (BMI) was 21.1 kg/m² (95% CI = 20.8–21.3 kg/m²).

Baseline characteristics of pregnant women and factors possibly associated with anemia are shown in Tables 2 and 3. Most (66.6%) women were illiterate, married (98.7%), or housewives (52.8%). More than half (54.0%) of them came from families who owned latrines.

A total of 68.3% were anemic and few (3.4%) had severe anemia. Iron deficiency anemia was prevalent in 24.3% of the pregnant women. A total of 15.1% had malaria parasites and 11.1% had intestinal helminths. Hookworms were the most prevalent species (80.9%). Almost 50% of the women had at least one nutritional deficiency. A total of 33.3% were iron deficient. Isolated deficiencies of iron (20.6%) and folate (18.8%) were most frequent. Only three (0.3%) women were deficient in iron, folate, and vitamin B12.

Factors associated with hemoglobin levels. Mean hemoglobin levels were lower in women less than 20 years of age and in primigravidae. Malaria infection, inflammatory syndromes, and nutritional and nutrient factors (low BMI and

TABLE 2

Characteristics of pregnant women at the time of first antenatal visit in Benin*

Characteristic	No. (%)
Illiterate	669 (66.6)
Married	992 (98.7)
Housewife	531 (52.8)
Having latrines	543 (54)
Having electricity	423 (42.1)
Having a refrigerator	52 (5.2)
Having a television	371 (36.9)
Having at least a bicycle	779 (77.5)
Primigravidae	190 (18.9)
Anemia (hemoglobin level < 110 g/L)	686 (68.3)
Mild anemia (hemoglobin level = 100–109 g/L)	324 (32.3)
Moderate anemia (hemoglobin level = 80–99 g/L)	328 (32.6)
Severe anemia (hemoglobin level < 80 g/L)	34 (3.4)
Iron deficiency	335 (33.3)
Folate deficiency	315 (31.3)
Vitamin B12 deficiency	36 (3.6)
Inflammatory syndrome	206 (20.5)
Malaria infection	152 (15.1)
Helminth infestations	110 (11.1)
Hemoglobin genotypes	
AA	725 (72.2)
AS	205 (20.4)
AC	61 (6.1)
Other (CC, SS, SC, AF)	13 (1.3)

* All no. = 1,005, except for hemoglobin genotypes (n = 1,004).

iron, folate, and vitamin B12 deficiencies) were associated with lower mean hemoglobin levels (Table 4). Having electricity in the house was associated with a higher hemoglobin concentration. There was no association between hemoglobin levels and hemoglobin genotypes, helminth infestations, intervals between pregnancies, and other socioeconomic characteristics.

Risk factors of anemia at the time of first ANV. Multivariate logistic regression showed that malaria infection; second trimester of gestation (gestational age ≥ 16 weeks); rainy season; low BMI; folate, iron, and vitamin B12 deficiencies; and helminth infestations were associated with a higher risk of anemia. Interestingly, odds ratios (ORs) were essentially unchanged after adjustment by the multivariate model, suggesting that the main risk factors were independently related to anemia in the study population (Table 5). Primigravidity (OR = 7.2, 95% CI = 3.5–14.7), folate deficiency (OR = 2.7, 95% CI = 1.3–5.5), and inflammatory syndrome (OR = 2.5,

TABLE 3

Distribution of helminth infestations and nutrient deficiencies in pregnant women at the time of first antenatal visit in Benin

Characteristic	No. (%)
Type of helminth infestation (n = 989)	
Hookworm	89 (9.0)
<i>Ascaris lumbricoides</i>	10 (1)
<i>Trichuris trichiura</i>	8 (0.8)
<i>Schistosoma mansoni</i>	2 (0.2)
Hookworm and <i>Ascaris lumbricoides</i>	1 (0.1)
Type of nutrient deficiencies (n = 1,005)	
Iron	207 (20.6)
Folate	189 (18.8)
Vitamin B12	15 (1.5)
Iron and folate	115 (11.4)
Iron and vitamin B12	10 (1.0)
Folate and vitamin B12	8 (0.8)
Iron, folate, and vitamin B12	3 (0.3)

TABLE 4

Factors associated with mean hemoglobin levels in 1,005 pregnant women in Benin, by univariate analysis*

Factor	No.	Mean (g/L)	95% CI	P
Age (years)				
≥ 20	748	104.0	103.2–104.8	
< 20	257	100.7	98.9–102.5	0.0002
Gestational age (weeks)				
< 16	80	109.7	106.9–112.5	
16–28	925	102.6	101.8–103.5	< 0.0001
Gravidity				
Primigravidae	190	99.3	97.1–101.4	
Multigravidae	815	104.1	103.3–104.9	< 0.0001
BMI (kg/m ²)				
≥ 20	560	104.5	103.5–105.6	
< 20	445	101.4	100.29–102.5	0.0001
Having electricity				
No	582	102.4	101.4–103.4	
Yes	423	104.2	103–105.3	0.026
Malaria				
No	853	104.1	103.3–104.9	
Yes	152	97.8	95.9–99.8	< 0.0001
Iron deficiency				
No	670	104.1	103.2–105.0	
Yes	335	101.3	99.9–102.6	0.0006
Folic acid deficiency				
No	690	104.1	103.2–105.1	
Yes	315	101.0	99.6–102.3	0.0002
Vitamin B12 deficiency				
No	969	103.3	102.5–104.1	
Yes	36	99.7	96.4–103.1	0.090
Inflammatory syndrome				
No	799	103.8	102.9–104.6	
Yes	206	100.8	99–102.48	0.0019
Season of inclusion				
Dry	602	104.2	103.2–105.2	
Rainy	403	101.6	100.4–102.7	0.0009

* There was no significant association between mean hemoglobin levels and the other socioeconomic characteristics, hemoglobin genotypes, helminth infestations, interval between pregnancies, and history of low birth weight. CI = confidence interval; BMI = body mass index.

95% CI = 1.2–5.3) were associated with a higher risk for severe anemia (hemoglobin level less than 80 g/L).

Population-attributable risks. A total of 21% (95% CI = 18–24%), 15% (95% CI = 13–17), 12% (95% CI = 10–14%), 11.0% (95% CI = 9–13%), 5% (95% CI = 4–6%), and 7% (95% CI = 5–9%) of anemia in this population were attributable to overall malnutrition, malaria infestation, ID, folate deficiency, vitamin B12 deficiency, and helminth infestations, respectively.

DISCUSSION

To our knowledge, this study is the first to investigate the risk factors of anemia during pregnancy before any preventive treatment. It is particularly important to obtain this information at the beginning of follow-up of a pregnant woman because medications given through the ANV package (sulfadoxine-pyrimethamine IPTp, iron and folic acid, anti-helminthics) can interact with physiologic variations of hemoglobin levels during pregnancy.^{4,19} Compared with previous studies,^{4,10,11} use of a wide range of variables potentially influencing the hemoglobin concentration on a large number of women (more than 1,000), and few missing data enabled us to perform a powerful analysis and to deal adequately with confounding factors.

Our results indicate that anemia is frequent in this population and that nutritional deficiencies (iron, folate, vitamin B12 deficiencies and more generally low BMI) and parasite infections (*P. falciparum* and intestinal helminths) are significant risk factors.

The finding of an increased prevalence of anemia is consistent with previous results from Benin and from other areas in sub-Saharan Africa.² More than 67% of the women had mild to moderate anemia. Moderate anemia may adversely affect physical performance^{2,20} and increase intrauterine growth retardation and risk of preterm delivery.^{21,22} With our hemoglobin cut-off of 80 g/L, 3.4% of pregnant women had severe anemia, supporting a previous finding in a neighboring area in Benin.⁴ The low proportion of women with a hemoglobin level less than 70 g/L in this study was similar to that found in neighboring countries.^{10,23}

In agreement with other studies in sub-Saharan Africa²⁴ and with a recent study in a neighboring area in Benin,⁴ malaria was one of the main factors associated with anemia. *Plasmodium falciparum* directly destroys erythrocytes,²⁵ but more complex phenomena are also involved, such as the inhibition of erythropoiesis by malarial pigment and malaria-induced pro-inflammatory mediators.²⁶ Fifteen percent of the women were infected at the first ANV, and 15% of anemic syndromes were attributable to malaria in our population. Such a relatively high prevalence is probably because the women had not been given the first IPTp dose when they were sampled and that for these women, the ANV was care-seeking oriented rather than motivated by a systematic follow-up of pregnancy. It could also partially explain the high proportion of inflammatory syndromes found in our population (more than 20%) because more than 33% of women with inflammation were malaria positive. Other infections (respiratory, genital, or urinary) could have contributed to increase the prevalence of inflammatory syndromes in this population. Unfortunately, because of the constraint of not interfering with the protocol of the clinical trial that our study was nested in, we were not able to investigate them more accurately.

Helminth infestations were also associated with an increased risk of anemia, as shown in other studies in tropical settings.^{27,28} In our population, 7% of anemia was attributable to helminth infestations. Hookworm infestations cause blood loss, putting the mother and the fetus at high risk for anemia. Considering the seriousness of these infections during pregnancy, the World Health Organization recommends that an antihelminthic treatment should be given to all pregnant women after the first trimester of gestation.²⁹ These recommendations are part of the national policy for antenatal prevention in Benin, and the women in this study were sampled before the administration of mebendazole.

For the first time in west Africa, we showed that ID increased the risk for anemia during pregnancy. Although iron requirements decrease in the first trimester because of the absence of menstruation, they increase steadily during the second trimester until the end of pregnancy.^{4,30} This augmentation is explained by the need to expand the plasma volume and erythrocyte mass of the mother,³¹ which are essential to development of fetal-placenta unit and because iron is a mandatory component of erythropoiesis.³² In sub-Saharan Africa, food taboos, inadequate dietary iron, and poor bioavailability of dietary iron from fiber adversely affect the storage of iron,

TABLE 5
Risk factors for anemia in 1,005 pregnant women in Benin, by univariate and multivariate analyses*

Factor	No.	Crude OR (95% CI)	P	Adjusted OR (95% CI)	P
Malaria					
No	853	1		1	
Yes	152	2.4 (1.6–3.7)	< 0.001	2.2 (1.4–3.5)	< 0.001
Gestational age (weeks)					
< 16	80	1		1	
≥ 16	925	2.1 (1.3–3.3)	0.002	1.7 (1.1–2.8)	0.025
Season of inclusion					
Dry	602	1		1	
Rainy	403	1.6 (1.2–2.1)	0.002	1.6 (1.2–2.1)	0.002
BMI (kg/m ²)					
≥ 20	560	1		1	
< 20	445	1.6 (1.2–2.1)	0.001	1.6 (1.2–2.2)	0.001
Folic acid deficiency					
No	690	1		1	
Yes	315	1.5 (1.1–1.9)	0.013	1.4 (1.0–1.9)	0.045
Iron deficiency					
No	670	1		1	
Yes	335	1.4 (1.0–1.8)	0.028	1.4 (1.1–1.9)	0.029
Helminth infestations					
No	879	1		1	
Yes	110	1.7 (1.1–2.7)	0.027	1.7 (1.0–2.7)	0.037
Vitamin B12 deficiency					
No	969	1		1	
Yes	36	2.4 (0.9–5.8)	0.055	2.4 (1.0–6.2)	0.049
Gravidity					
Muligravidae	815	1		–	
Primigravidae	190	1.3 (0.9–1.8)	0.2	–	–
Possession of latrines					
No	462	1		–	
Yes	543	0.8 (0.6–1.1)	0.11	–	–
Inflammatory syndrome					
No	799	1		–	
Yes	206	1.3 (0.9–1.8)	0.12	–	–
Age (years)					
> 20	748	1		–	
≤ 20	257	1.3 (0.9–1.7)	0.14	–	–

* CI = confidence interval; BMI, body mass index.

eventually causing anemia. Iron deficiency is usually considered to be the leading cause of anemia in pregnant women. Although we showed that ID was an important cause of anemia in our population, it represented only 12% of the attributable causes, after overall malnutrition and malaria.

We used serum ferritin concentrations to assess the iron store of pregnant women and CRP (a marker of the acute phase of infection) to interpret values in cases of inflammation. The measurement of alpha 1 acid glycoprotein, which seems to be the most appropriate in correcting thresholds of serum ferritin in the context of chronic inflammation,¹³ was not technically and financially feasible at the time our study was conducted. Therefore, the effects of chronic inflammation on serum ferritin levels are likely to have been underestimated.³³ In theory, the best technique for diagnosing IDA is the examination of stained bone marrow aspirates for storage iron as hemosiderin.³⁴ However, this method is ethically unacceptable in a population survey, especially in apparently healthy pregnant women.

Other indicators such as transferrin and transferrin saturation could have been used to assess iron status. However, their interpretation can be affected by several factors such as intake of meals, malignant syndromes, liver diseases, nephritic syndrome, and malnutrition.^{35,36} Furthermore, the day-to-day variation of transferrin saturation is less sensitive to iron deficiency than is the serum ferritin concentration

because changes in transferrin saturation occur after iron stores are depleted.³⁷

In accordance with a study in Malawi,¹¹ folate deficiency was common in this population and was associated with an increased risk of anemia. These findings may have been caused by dietary insufficiency, absorption disorders, and parasitic or infectious diseases. With a cut-off value of 150 pg/L, low vitamin B12 concentrations were found in 3.6% of the women. This deficiency is commonly believed to induce megaloblastic anemia. In our study, it was associated with an increased risk of maternal anemia as found in other areas in Africa.³² Independently from iron, folic acid, and vitamin B12 deficiencies, a low BMI (indicating overall malnutrition) was also associated with anemia. At a population level, more than 20% of anemia cases were attributable to overall malnutrition. This finding stresses the role that other micronutrients deficiencies may play in the occurrence of anemia during pregnancy.

More than 44% of women in this population experienced malnutrition at the beginning of pregnancy. This finding is probably caused by effects of food restrictions in pregnant women, which are common and described as important underlying causes of malnutrition and anemia in Africa and elsewhere.³⁸ As Ayoya and others have reported, several vitamin- and mineral-rich foods (eggs, meat, and milk) are seldom consumed or their consumption is limited because of the belief that they may result in poor pregnancy issues. Financial

limitations also commonly hamper access to animal proteins and other more expensive mineral and vitamins rich-foods.¹⁰ It has been found in a study identifying dietary patterns of urban adults in Benin that intakes of vitamin E, vitamin B12, calcium, and zinc were overall low.³⁹ Moreover, unequal household distribution of vitamins and protein-rich foods favoring men over women and children further exacerbates this problem. Unfortunately, the original design did not include micronutrients other than iron, folic acid and vitamin B12, and this may be a limitation to the study.

Most women in our sample had an AA hemoglobin genotype and almost 21% had heterozygous AS. Only one woman had sickle cell disease, and this was probably because of the fact that most women with an SS hemoglobin genotype did not reach the age of childbearing. Hemoglobin SS-associated high rates of mortality can be related to low levels of access to care, delay in the diagnosis of the disease, or the result of ineffective treatment. We did not observe any association between anemia and heterozygous AS genotype. In sub-Saharan Africa, sickle cell disease, although a serious disease, is not a common problem in pregnant women. It is likely that improved health services will enable an increasing number of these patients to reach reproductive age and that the role played by genetic abnormalities of hemoglobin will become more important.

In addition to *P. falciparum* and helminth infestations, genital and urinary infections have been suggested to be associated with an increased risk for anemia in pregnant women.^{10,11} However, being chronic and associated with mild symptoms (if any), they are unlikely to be promptly reported by women. They are believed to act through inflammation, impairing erythropoiesis, and interfering with mobilization of reticulo-endothelial iron storages,⁴⁰ and shortening erythrocyte survival. However, because our study was nested in a multicenter clinical trial, there was no possibility to actively search for asymptomatic infections without treating them and thus inducing a bias between trial sites.

Anemia in pregnancy is common in Benin, and in addition to malaria infection, nutritional factors (particularly iron and folic acid) and helminth infestations are its main causes. Most of these potential causes can be prevented by using existing tools such as IPTp, antihelminthic treatment, and iron and folic acid supplementation, which have been administered to the women after they were sampled. By following-up this population until delivery, we will be able to assess the impact of prevention strategies on the persistence and severity of anemia.

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