Malaria Is More Prevalent Than Iron Deficiency among Anemic Pregnant Women at the First Antenatal Visit in Rural South Kivu

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Abstract. Anemia is common during pregnancy and is associated with poor outcomes. Objectives were not only 1) to determine the prevalence of anemia and iron deficiency (ID) but also 2) to identify other factors associated with anemia in pregnant women from South Kivu province, in the eastern Democratic Republic of Congo. Between December 2013 and March 2014, 531 women attending the first antenatal visit in their second trimester of pregnancy were recruited. Sociodemographic, clinical, and biological data were collected. Hemoglobin (Hb) was determined by a portable photometer (Hemocue® Hb201+), and anemia was defined as altitude-adjusted Hb < 110 g/L. ID was defined as serum ferritin < 15 µg/L adjusted for inflammation status (C-reactive protein [CRP] > 5 mg/L and/or α-1-acid glycoprotein > 1 g/L) whereas hypoalbuminemia was defined as serum albumin < 35 g/L. A Giemsa-stained blood smear was used to diagnose malaria. The median age (interquartile range) was 25.5 (21.1–31.3) years, with anemia in 17.6% and ID in 8%. Malaria was present in 7.5% and hypoalbuminemia among 44%. Soluble transferrin receptor concentration was higher in the presence of inflammation and/or malaria. In the final logistic regression model, factors independently associated with anemia were malaria (adjusted odds ratio [aOR]: 11.24 (4.98–25.37) $P < 0.001$), hypoalbuminemia [aOR: 2.14 (1.27–3.59); $P = 0.004$] and elevated CRP [aOR: 1.94 (1.10–3.45); $P = 0.022$]. ID was not highly prevalent and not associated with anemia in our population. Effective control of anemia during pregnancy in this region should consider fighting malaria and other infectious diseases in combination with measures to improve women's nutrition, both before and during pregnancy.

INTRODUCTION

It is estimated that a quarter of the world's population suffers from anemia,¹ and it has been established that pregnant women are one of the most affected groups, particularly in low-income countries.² According to a study published in 2008, which summarized global data on anemia for the period 1993–2005, 55.8% of pregnant women in Africa were anemic.¹ Another more recent publication, which compared anemia trends worldwide between 1995 and 2011, reported a reduction in the prevalence of anemia at the global level and showed again the heterogeneity of the problem of anemia according to the different regions of the globe.³ Between 1995 and 2011, the overall prevalence of anemia decreased from 43% to 38%. In Eastern Africa, this prevalence decreased from 46% to 36%, and in Central and Western Africa, it decreased from 61% to 56%.³

The 2007 Democratic Republic of Congo (DRC) demographic and health survey (DHS) estimated a 53% national prevalence of anemia with a 39% regional prevalence in South Kivu province among women of child-bearing age.⁴ Furthermore, the 2007 DRC DHS estimated the national prevalence of anemia at 60% among pregnant women, but the report lacked this data for individual provinces. According to the World Health Organization (WHO), this level of prevalence, at national level, is considered to be a severe public health problem.⁵

Anemia during pregnancy is known to be associated with a high risk of poor birth outcomes.⁶ It causes fatigue, reduces work capacity,⁷ and is associated with a high risk of maternal mortality^{6,8}; which remains a real public health problem with a 2015 global estimate of 300,000 maternal deaths.⁹ In anemic (and undernourished) women, even minimal blood losses can have serious consequences; complications of postpartum hemorrhage are higher in the presence of preexisting anemia.¹⁰ Hemorrhage tops among the leading five clinical conditions responsible for at least 80% of maternal death.¹¹ Geller et al.¹² estimates that postpartum hemorrhage accounts for 25–33% of all maternal deaths.

The etiology of anemia is multifactorial; the common causes being of nutritional origin (such as iron deficiency [ID]), infections, and genetic disorders.^{2,13} These causes may vary between different settings and may be synergistic at varying degrees in different settings. WHO estimates that half of all cases of anemia are related to ID.⁶

Despite the high burden of anemia in DRC, there are limited data on its etiology among pregnant women and especially the relative contribution of ID. In 1994, Kuvibidila et al.¹⁴ found a higher prevalence of ID in the western province of Kongo Central (formerly Bas-Congo) among pregnant women than nonpregnant women and a control group of nonpregnant Caucasian women living in the United States of America. The study used ferritin or adjusted ferritin in case of inflammation to evaluate iron store, and it was generally found that Congolese women had a lower mean plasma iron as compared with the control group regardless of their physiological status.¹⁴ In his 1996 article from the same region, Kuvibidila and colleagues¹⁵ reported a 56% ID prevalence among pregnant women without inflammation as determined by a serum ferritin (SF) < 12 µg/L and/or transferrin saturation < 16%. It should be noted that

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these results could not be generalized to the entire country owing to the different sociodemographic, geographic, genetic, and behavioral differences (including eating habits) between different ethnic groups and regions.

The 2007 DHS used hemoglobin (Hb) alone as a proxy for ID on the assumption that ID contributes to half of all cases of anemia. This was an oversight in determining the prevalence of ID considering that causes of anemia are diverse and even synergistic in some individuals, more specifically pregnant women. There has been no documented study carried out using validated biochemical parameters for iron store evaluation among pregnant women in Kivu. This study aimed at bridging this information gap with the following objectives: 1) to determine the prevalence of anemia and that of ID and 2) to identify other factors associated with anemia among pregnant women from the rural Miti-Murhesa health zone in South Kivu province in eastern DRC.

MATERIALS AND METHODS

Study settings. The study was conducted in Miti-Murhesa health zone, a rural area in the northeastern part of the South Kivu Province in eastern DRC. It is located between 1,500 and 2,000 m above sea level, and in 2013, it had a population estimated at 250,000 inhabitants. The main activity of the population in this region is subsistence agriculture with a diet low in products of animal origin. In a survey done in 2010–2011, the prevalence of malaria among pregnant women at the first antenatal care (ANC) visit in the second trimester of pregnancy was 9.5%.¹⁶

Study participants were enrolled at the Miti-Murhesa general referral hospital, a secondary health facility level that offers preventive care such as ANC and curative care to patients referred by surrounding primary health centers. The hospital mostly serves people from the southern part of the Miti-Murhesa health zone because the northern part has another health facility with the same level of service provision.

Study design and sample size. Between December 2013 and March 2014, pregnant women were recruited in a cross-sectional study from women attending ANC visits. This period includes both low and high malaria transmission periods.¹⁶ The sample size was determined considering a prevalence of anemia of about 32% (E. Bahizire, unpublished data from a survey done in the same area in 2010–2011), a confidence interval (CI) of 95%, a precision of 5%, a nonresponse, and/or concern with blood drawn rate of 20%; so, the minimum sample size required for this study was 334 subjects. Finally, 531 pregnant women were enrolled in the study.

Recruitment and study population. Informed consent was sought in the local language from eligible women attending ANC visits. Study participants were all consenting pregnant women living in the Miti-Murhesa health zone at the first antenatal visit during their second trimester of pregnancy (between 12 and 24 weeks of gestational age). During the study period, 1,239 women attended their first ANC visit. Among them, around 66% were in the second trimester according to the last menstrual period and/or fundal height. But finally, we only enrolled those for whom the last menstrual period was known. Among eligible women, almost 98% accepted to participate in the present study. Women testing reactive to the human immunodeficiency virus (HIV) serology test were excluded.

Data collection and procedures. A standard pretested questionnaire was administered in the local language by trained nurses. The questionnaire included basic demographic

questions regarding age, marital status, education level, employment, household size, and obstetrical history of the women. General background information on febrile morbidity and the history of taking any medications within the 3 months before the visit was recorded. The treatment history focused on antimalarial drugs (quinine, sulfadoxine-pyrimethamine, and artemisinin-based combination therapy). Iron and/or folic acid prescription and deworming treatment (mebendazole and albendazole) were also noted. The questionnaire enquired further on the use of the insecticide-treated mosquito net (ITN) on the night before the interview as a proxy for regular ITN use.

Mid-upper arm circumference (MUAC) was measured following the Food and Nutrition Technical Assistance guidelines.¹⁷ Measurements were determined from the mean of two separate measurements, and a tiebreaker was only done when the difference between the first two measurements was outside a predetermined acceptable limit (0.5 cm).¹⁷

Blood collection and laboratory methods. Hb was measured on site using a portable HemoCue Hb201⁺ point-of-care analyzer (HemoCue AB, Ångelholm, Sweden). A venous blood sample was drawn by venipuncture and stored in a collection tube inside a cool box containing dry ice while in the field. The blood was allowed to settle and serum pipetted out at the end of the day and stored at –40°C until shipment. Part of the serum was used for the determination of albumin concentration using spectrophotometer GENESYS 20 (Thermo Fisher Scientific, Waltham, MA). The frozen samples were transported in a styrofoam box with dry ice to “VitMin Lab” (Willstaett, Germany) where the sandwich enzyme-linked immunosorbent assay (ELISA) technique was used to determine SF, soluble transferrin receptor (sTfR), retinol-binding protein (RBP), C-reactive protein (CRP), and α -1-acid-glycoprotein (AGP).¹⁸ A quality control check was conducted, and coefficients of variation (CV) were found to be within recommended range, (calculated CV were: 3.01% for ferritin, 4.58% for sTfR, 4.21% for RBP, 6.55% for CRP, and 5.96% for AGP).

Malaria diagnosis was made on a thick blood smear stained with a Giemsa 3% for 30 minutes according to the WHO guidelines. *Plasmodium* species identification was made on a thin smear. Malaria was defined as the presence of the asexual *Plasmodium* stage regardless of parasite density. A thick blood smear was considered negative for malaria after reading at least 100 microscopic fields. Quality control was ensured by sending 10% of randomly selected slides for review to an independent experienced laboratory technician.

HIV testing was done routinely on all pregnant women according to WHO and national DRC guidelines. Two of the eligible women were excluded on the basis of a positive result from the routine HIV serology test.

Variables transformation. The ages of the study participants were categorized into the following three age groups: ≤ 20 years, > 20 –35 years, and > 35 years. Literacy level was summarized into the following categories using the formal education system as a proxy: the lowest (no school) included women with no formal education and those who only went up to primary school, the second (primary) included those who completed primary school but did not complete high school; and the third (secondary) included all those who at least completed secondary school. Employment class was categorized as follows; the first group included all those responding that they were not used whereas the second group included those who declared that they were farmers (small farming), and the third group named “other” included women with all other forms of employment.

In this study, the MUAC was dichotomized with the threshold value proposed by Ferro-Luzzi et al.¹⁹ for adult women; thus, undernutrition was defined as MUAC < 220 mm.

SF as an acute phase protein is known to increase during infection/inflammation.²⁰ Therefore, the SF was adjusted according to an individual's inflammation status, based on CRP and AGP values (CRP > 5 mg/L and/or AGP > 1 g/L). Adjustment was done according to inflammation steps as suggested by Thurnham (no inflammation, incubation [elevated CRP only], early convalescence [both CRP and AGP elevated] and late convalescence [elevated AGP only]).²¹ For this study, ID was defined as low SF concentration using standard cut offs with inflammation-adjusted SF < 15 µg/L. When sTfR was used to evaluate iron load, ID was defined as sTfR > 8.3 mg/L. RBP was adjusted for inflammation using corrections proposed by Thurnham et al.²² Vitamin A deficiency was defined as inflammation-adjusted RBP < 0.7 µmol/L. Because all villages were more than 1,500 m above sea level, Hb concentration was adjusted accordingly, and anemia was defined by Hb concentrations of < 110 g/L and was defined as severe when < 70 g/L according to the WHO guidelines.²³ Hypoalbuminemia was defined as serum albumin < 35 g/L.²⁴

Data management and statistical analysis. Data were double entered using Microsoft Access 2007 (Microsoft Corporation, Redmond, WA), and statistical analyses were performed using STATA for Mac, version 12.1 (StataCorp, College Station, TX). Participant descriptive characteristics were summarized as mean and standard deviation for normal continuous variables, or as median and interquartile range for nonnormal continuous variables, and finally, as percentages for categorical variables. Pearson's chi-square or Fisher's exact test were used to examine bivariate relationships between variables, and also, odds ratio and 95% CI were used. Logistic regression model after stepwise backward removal was used to examine the independent association between different factors and anemia, the dependent variable. For all analysis, the statistical significance level was fixed at < 0.05.

Ethical aspects. The study was approved by the local Institutional Ethics Committee of the Catholic University of Bukavu and was authorized by the Ministry of Health office in the South Kivu province. All participants gave an informed consent, and the women excluded based on the positive serology HIV test were managed according to the national guidelines. All data were managed in a manner to guarantee confidentiality for all participants.

RESULTS

Sociodemographic data, information on morbidity, clinical data, and biological parameters of the study participants are summarized in Table 1. Almost all the study participants were married with 70% of them being in the 20–35 years age group. Half of the study population was in their 4th pregnancy while 72% were not educated. Almost 4 of 10 reported fever within the past 3 months and 9% within the 2 days before the interview. Responses on drug history indicated that only 1% received folic acid and 2% iron tablets whereas 6% reported antihelminths treatment, and less than 2 of 10 reported anti-malaria treatment within the past 3 months. More than 6 of 10 women reported sleeping under ITN the prior night. About nutritional status, less than 5% had a low MUAC (< 220 mm) and slightly less than 2% had vitamin A deficiency (RBP <

0.7 µmol/L) whereas nearly half of women presented hypoalbuminemia. The prevalence of malaria was 7.5%. Among the 40 malaria-positive women, 38 had *Plasmodium falciparum* and two had *Plasmodium malariae*. Inflammation was present in almost a quarter of the subjects.

The prevalence of anemia was 17.6% (95% CI, 14.4–21.1) with 0.2% of severe anemia (*N* = 529). ID determined by adjusted ferritin was present in 7.6% (95% CI, 5.4–10.4; *N* = 485) of the study population and iron deficiency anemia was 9.4% (*N* = 85). Among women without inflammation (*N* = 369), the prevalence of ID, evaluated by unadjusted ferritin, was 7.3%. The prevalence of ID is almost the same between noninflamed subjects using unadjusted ferritin and the entire population including those inflamed, using adjusted ferritin. ID was found to be 12.4% (95% CI, 9.6–15.6; *N* = 485) when sTfR was used to evaluate iron load. The sTfR prevalence of ID appeared to rise in the presence of inflammation or malaria, being 10.6% (*N* = 329) and 18.1% (*N* = 116), respectively, in women without and with inflammation and also being 10.5% (*N* = 449) and 37.1% (*N* = 35), respectively, in the absence and in the presence of malaria (Figure 1).

Bivariate analyses of anemia with other factors are presented in Table 2 and Table 3. It was found that anemia was associated with malaria, hypoalbuminemia, and inflammation either based on the CRP or the AGP levels. Anemia was associated with ID when evaluated by sTfR but not when ferritin was used. Table 4 displays results of a multivariable analysis. In the first model where ID was evaluated by SF, ID was not associated with anemia but in the second model where ID was evaluated by sTfR, ID was independently associated with anemia. In the two models, malaria, hypoalbuminemia, and CRP were independently associated with anemia.

DISCUSSION

Our results suggest that anemia in pregnant women from the Miti-Murhesa health zone (17.6%) is a public health problem, which can be classified as mild according to the WHO classification (prevalence < 20%) for this group.⁵ ID was present in 7.6% of the population using SF indicator but slightly higher, 12.4%, when sTfR indicator was used. The prevalence of IDA (IDA) among pregnant women was found in 9.4% using the SF indicator and in 27.1% when sTfR indicator was used (Figure 1). In a multivariable analysis, malaria low serum albumin and raised CRP were each found to be independently associated with anemia when SF was used as an indicator of iron status. When sTfR was used to evaluate iron load, ID was independently associated with anemia in addition to the above three variables.

Despite being the first to evaluate the relationship between anemia and ID in pregnant women from the Miti-Murhesa health zone, the present study presents some methodological limitations. Firstly, our results might not be representative of all pregnant women from the study area because of the sampling method used. This might underestimate the prevalence of anemia and even that of ID as women who did not attend ANC could be at higher risk (less educated and undernourished). It has been reported that less privileged women are less likely to access ANC services.²⁵ Secondly, the cross-sectional design cannot establish any causal relationship between anemia and the studied factors (ID and other associated factors). Finally, we did not investigate other possible causes of anemia such as hemoglobinopathies, genetic abnormalities, and the presence of helminths.

TABLE 1
Demographic clinical and biological characteristics of pregnant women

Variables	n	%	Mean (SD)	Median (P25–P75)
Age (years)	530	—	—	25.5 (21.1–31.3)
≤ 20	—	17.9	—	—
> 20–35	—	68.9	—	—
> 35	—	13.2	—	—
Marital status (% married)	531	96.8	—	—
Number of pregnancy	531	—	—	—
1	—	26.4	—	—
2–3	—	22.4	—	—
≥ 4	—	51.2	—	—
Level of education	531	—	—	—
None	—	71.9	—	—
Primary	—	21.9	—	—
Secondary	—	6.2	—	—
Activities/employment	529	—	—	—
None	—	15.8	—	—
Cultivator (small farming)	—	60.8	—	—
Other	—	23.4	—	—
Mid-upper arm circumference (mm)	529	—	258.9 (25.6)	—
< 220	—	4.2	—	—
Fever within the last three months	530	37.5	—	—
Fever within the last 48 hours	525	9.0	—	—
Sleeping under ITN* the night prior	527	63.8	—	—
Deworming during the last 3 months	511	6.1	—	—
Folic acid supplementation within the last 3 months	521	1.0	—	—
Iron supplementation within the last 3 months	521	2.1	—	—
Antimalarial treatment within the last 3 months	522	16.5	—	—
Biological parameters				
Malaria	530	7.5	—	—
Hemoglobin adjusted for altitude (g/L)	529	—	121.2 (14.0)	—
< 70	—	0.2	—	—
70–109	—	17.4	—	—
≥ 110	—	82.4	—	—
Serum albumin (g/L)	510	—	35 (6)	—
< 35	—	44.1	—	—
Soluble transferrin receptor (mg/L)	485	—	—	5.7 (4.7–7.0)
> 8.3	—	12.4	—	—
Ferritin (μg/L), adjusted for APP	485	—	—	41.4 (24.5–64.6)
< 15	—	7.6	—	—
15 to < 30	—	24.1	—	—
≥ 30	—	68.3	—	—
Iron deficiency (ID, ferritin adjusted for APP < 15 μg/L)	485	7.6	—	—
ID among noninflamed (unadjusted ferritin < 15 μg/L)	369	7.3	—	—
C-reactive protein (CRP mg/L)	485	—	—	1.9 (0.7–4.3)
> 5	—	22.5	—	—
α1-acid glycoprotein (AGP g/L)	485	—	—	0.52 (0.4–0.7)
> 1	—	7.0	—	—
Retinol binding protein adjusted for APP (μmol/L)	485	—	1.7 (0.5)	—
Vitamin A deficiency (< 0.7)	—	1.7	—	—
Inflammation†	485	23.9	—	—

* ITN = insecticide-treated mosquito net.

† CRP > 5 mg/L and/or AGP > 1 g/L; APP = acute phase proteins (CRP and AGP).

Prevalence of anemia. The prevalence of anemia in our study was 17.6% (14.4–21.1). This figure is lower than the 60% reported among pregnant women by the 2007 DHS at the national level⁴ and less than the estimate of anemia in Central and Western Africa (56%) on one hand and Eastern Africa (36%) on the other hand.³ Although the 2007 DRC DHS did not report the prevalence of anemia in pregnant women by province, we assume that it was more than 38%,⁴ which was the prevalence of anemia among women of childbearing age (WCBA) from South Kivu, as it is known that usually the prevalence of anemia is higher among pregnant women as compared with nonpregnant women.^{2,5} However, the level of anemia in the present study was close to 16.5% (13.2–20.3) reported in a recent survey done in the same province including the same health zone in 2014 among nonpregnant

WCBA.²⁶ In the same health zone, another study conducted in 2010–2011 reported a prevalence of anemia among pregnant women of about 32% (E. Bahizire, unpublished data). Comparison of anemia prevalence between the 2007 DHS⁴ and that of this recent survey in South Kivu²⁶ in WCBA but nonpregnant shows a declining trend in the prevalence of anemia among pregnant women in the Miti-Murhesa health zone. This figure has been reported elsewhere in other countries.³

The decreased prevalence of malaria, evident in pregnant women from the Miti-Murhesa health zone, comparing 2010–2011 and 2013–2014 can explain part of the decrease of anemia prevalence. Indeed, the global prevalence of malaria was 9.5% in a survey conducted in the Miti-Murhesa health zone in 2010–2011.¹⁶ Two health facilities were used

FIGURE 1. Proportion of pregnant women with high level of soluble transferrin receptor (sTfR)* according to anemia, inflammation, and malaria. Comparisons between § and #: $P = 0.365$; between § and €: $P = 0.020$ (Fisher exact); between § and \$: $P < 0.001$.

for the recruitment of pregnant women in the survey but the precise prevalence of malaria among pregnant women enrolled at the Miti-Murhesa general referral hospital was 14.5% (E. Bahizire, unpublished data). In the present study, we report a prevalence of malaria of about 7.5%. This picture of reduced anemia prevalence in relation to decreased malaria prevalence has also been reported elsewhere among preschool children.^{27,28} The decline in malaria prevalence followed public health activities to combat malaria on a large scale including ITN distribution, increased use of malaria rapid diagnostic tests, and artemisinin-combination therapy for the treatment of malaria cases in the region.¹⁶

The 2014 DRC DHS compared with 2007 DHS showed a slight improvement in the nutritional status of WCBA from South Kivu who had a body mass index (BMI) < 18.5 from 9.2% to 7.2%.^{4,29} Although, we don't have the specific picture for the Miti-Murhesa health zone, but we would suggest that nutritional improvement might explain part of the decrease in anemia prevalence. We appreciate the possibility of other factors contributing to the decrease in anemia burden but there is a lack of published data on causes or risk factors that were associated with anemia in pregnant women from this region. There is also a lack of data on the trends of known causes of anemia in our study area. We can only postulate that contribution of hemoglobinopathies to anemia has not changed over time, and it is difficult to comment on the contribution of helminths infections to anemia. We do not think that HIV could explain the low prevalence of anemia in the present study compared with 2014 DRC DHS, as HIV prevalence was reported to be low in South Kivu (0.8% among women between 15 and 49 years) by the 2014 DRC DHS.²⁹ It should also be noted that there were no public health interventions to address anemia such as food fortification, iron supplementation, or deworming program for adults in this community at the time of the study.

Nutritional deficiencies-hypoalbuminemia and ID.

Anemia was more prevalent in women with low serum albumin. Nutritional deficiencies are known to cause anemia.^{2,13} From immature proerythroblasts to mature red blood cells (RBC), the body undergoes multiple cellular divisions and protein synthesis. The globin, protein part of Hb, needs amino acids to be synthesized; thus, a deficiency in amino acid may lead to a reduction in the formation of Hb thereby impairing erythropoiesis.³⁰

As it has been reported that nutritional deficiencies (macro and micronutrients) are often combined¹³ and that zinc deficiency is highly prevalent (51.8%) in the region among non-pregnant WCBA,²⁶ zinc deficiency might be associated to the high proportion of hypoalbuminemia and could in turn explain part of the anemia burden in our study population. This gap calls for further research to understand its true contribution to anemia in the region. People with zinc deficiency may present oxidative stress that can induce anemia by the reduction of RBC lifespan.³¹ And, as zinc is involved in erythropoiesis and plays a role as an iron metabolism catalyst,³² its deficiency may be associated with anemia.

In our study population, anemic subjects had a lower than 50% ID (as suggested by WHO) regardless of the parameter used to evaluate iron load; SF adjusted for inflammation (9.4%) or sTfR (27.1%). ID was positively and independently associated with anemia only when using sTfR but not when using SF. Indeed, ferritin concentration evaluates iron stores whereas sTfR is an indicator of iron deficient erythropoiesis (functional tissue ID).³³ Logically, iron stores should be finished before erythropoiesis become iron deficient. But unexpectedly, similar conflicting results (higher level of iron deficient erythropoiesis than deficient iron store) have been reported by other published articles, in our study region²⁶ and in other settings.³⁴

It has been reported that hemoglobinopathies,^{35,36} and malaria^{37,38} raise sTfR even in the absence of ID which may

TABLE 2
Demographic and clinical risk factors of anemia in pregnant women

Variables	n	% Anemia	OR (95% CI)	P
Age (years)	—	—	—	0.270
≤ 20	95	17.9	1.12 (0.62–2.03)	—
> 20–35	363	16.3	1	—
> 35	70	24.3	1.65 (0.89–3.05)	—
Marital status	—	—	—	0.994
Single	17	17.7	1.00 (0.28–3.57)	—
Married	512	17.6	1	—
Number of pregnancy	—	—	—	0.587
1	140	20.0	1.4 (0.73–2.68)	—
2–3	119	15.1	1	—
≥ 4	270	17.4	1.18 (0.65–2.13)	—
Level of education	—	—	—	0.374
None	380	17.6	2.14 (0.63–7.22)	—
Primary	116	19.8	2.47 (0.69–8.82)	—
Secondary	33	9.1	1	—
Activities/Employment	—	—	—	0.531
None	84	20.2	1.49 (0.72–3.10)	—
Cultivator (small farming)	321	18.1	1.30 (0.73–2.30)	—
Other	124	14.5	1	—
Mid-upper arm circumference (mm)	—	—	—	0.946
< 220	22	18.2	1.04 (0.34–3.14)	—
≥ 220	505	17.6	1	—
Fever within the last three months	—	—	—	0.461
No	330	16.7	1	—
Yes	198	19.2	1.19 (0.75–1.88)	—
Fever within the last 48 hours	—	—	—	0.273
No	476	17.0	1	—
Yes	47	23.4	1.49 (0.72–3.04)	—
Sleeping under insecticide-treated mosquito net the night prior	—	—	—	0.220
No	191	20.4	1.33 (0.84–2.13)	—
Yes	334	16.2	1	—
Deworming during the last 3 months	—	—	—	0.801
No	478	17.6	1	—
Yes	31	19.4	1.13 (0.37–2.93)	—
Antimalarial treatment during the last 3 months	—	—	—	0.725
No	435	17.2	1	—
Yes	85	18.8	1.11 (0.57–2.07)	—

CI = confidence interval; OR = odds ratio.

confuse the interpretation of iron load in a setting where these conditions are present. There are fewer consensuses on the fact that the inflammation influences sTfR level and whether an adjustment should be applied to correct sTfR concentration according to an individual inflammation status. It has been suggested that sTfR could be more reliable in the presence of inflammation.^{20,33} In Figure 1, we report that sTfR was increased in presence of malaria with or without elevated acute phase proteins, $P < 0.001$ and $P = 0.020$ (Fisher exact) respectively. This figure has been previously reported.^{37,38} Our results highlight also the fact that sTfR was increased in the presence of inflammation but this difference was not significant. This finding is in accordance with previously published data,^{20,33} but contrasts with results from a study carried out in Côte d'Ivoire by Righetti and colleagues,³⁹ who reported that elevated sTfR was significantly associated with inflammation. Around 70% of the malaria infected subjects had elevated acute phase proteins although we cannot confirm what proportion of inflammation was associated with submicroscopic malaria in our population. About malaria infection, one should note that submicroscopic malaria infection is common during pregnancy,^{40–42} meaning that malaria could be more prevalent than what was reported by microscopy in our population. These findings suggest that there is a need for correction factors for sTfR to be used in an endemic malaria settings and

that sTfR concentration should be interpreted with caution in a population where infections/inflammation are prevalent. The need for methods to adjust sTfR for inflammation has been highlighted by Suchdev et al.⁴³ In Kivu, some data suggest hemoglobinopathies might be more prevalent than what has been previously thought. Indeed, a report from Kinshasa, the capital city of DRC, has shown a prevalence of about 14.4% sickle cell trait among new-born babies of mothers originating from Kivu during a screening for this condition.⁴⁴ In Rwanda, a country sharing a border with South Kivu province, α^+ -thalassemia has been reported in 15.1% of preschool children in a community survey.⁴⁵ In a recent study in South Kivu (in which some of us are involved) among preschool children, sickle cell trait, $\alpha^{3.7}$ -thalassemia, and glucose-6-phosphate dehydrogenase A-deficiency were observed in 6%, 12%, and 17%, respectively (C. D. Karakochuk et al., unpublished data). We suggest that sTfR might not be a good indicator of iron load in this region in the absence of validated corrections that will take into account these other conditions and/or comorbidities that influence its levels regardless of iron load.

According to our results, it seems that ID might not contribute highly to the burden of anemia in pregnant women from the Miti-Murhesa health zone. Similar results have been reported among WCBA and preschool children from the region.^{26,46} It is important to identify other etiologies of anemia

TABLE 3
Biological risk factors of anemia in pregnant women

Variables	n	% Anemia	OR (95% CI)	P
Malaria	—	—	—	< 0.001
No	488	13.5	1	—
Yes	40	67.5	13.28 (6.21–29.30)	—
Serum albumin (g/L)	—	—	—	0.003
< 35	223	23.8	1.96 (1.21–3.20)	—
≥ 35	285	13.7	1	—
Iron deficiency—ferritin (adjusted for APP)	—	—	—	0.504
Yes	37	21.6	1.32 (0.58–3.00)	—
No	446	17.3	1	—
Iron deficiency—sTfR	—	—	—	< 0.001
Yes	60	38.3	3.62 (1.91–6.73)	—
No	423	14.7	1	—
C-reactive protein (CRP mg/L)	—	—	—	< 0.001
≤ 5	374	13.4	1	—
> 5	109	32.1	3.06 (1.79–5.19)	—
α1-acid glycoprotein (AGP g/L)	—	—	—	0.005
≤ 1	449	16.3	1	—
> 1	34	35.3	2.81 (1.21–6.22)	—
Retinol binding protein adjusted for APP (μmol/L)	—	—	—	0.136
Deficiency (< 0.7)	8	37.5	2.88 (0.44–15.07)	—
Nondeficiency (≥ 0.7)	475	17.3	1	—
Inflammation*	—	—	—	< 0.001
No	367	13.6	1	—
Yes	116	30.2	2.74 (1.61–4.62)	—

APP = acute phase proteins CRP and AGP; CI = confidence interval; OR = odds ratio; sTfR = soluble transferrin receptor.

* CRP > 5 mg/L and/or AGP > 1 g/L.

in the South Kivu province and in the entire country or even the whole region to design more effective interventions. Etiology might be context specific. These inherited disorders might contribute more than ID to the occurrence of anemia in our population. A possible explanation of the low level of ID can be the presence of ferroportin Q248H mutation associated with hemochromatosis type IV (autosomal dominant hereditary iron overload disease). This mutation has been reported in 14% of subjects from South Kivu.⁴⁷ Another possible explanation might be the high concentration of iron in drinking water (from ground water), as the volcanic soil of Kivu is known to be rich in iron. This possibility has been suggested in Prey Veng province in Cambodia⁴⁸ and recently, has been reported in Bangladesh where ID was less prevalent in the region where the soil is rich in iron.⁴⁹

Our findings of a lower ID prevalence are similar to those from a study conducted among nonpregnant WCBA in the region²⁶ and to another study from Cambodia,³⁴ showing that IDA was not prevalent. In Benin, Ouédraogo et al.⁵⁰ reported that among pregnant women at their first antenatal visit, anemia was more prevalent in women with malnutrition (macronutrient and micronutrient), including BMI < 20, ID, vitamin B12, and folic acid deficiencies. Even if during pregnancy there is much nutritional demand, in our population, it might not be the same figure as it has been shown that these deficiencies are not prevalent in nonpregnant WCBA from South Kivu,²⁶ but as previously said, there is a need to further study this relationship in pregnant women.

Malaria and raised CRP/inflammation. Malaria was the factor strongly associated with anemia in pregnant women in

TABLE 4
Logistic regression and adjusted odd ratio of anemia in pregnant women

Variables	Model 1,* N = 482; 85 cases of anemia		Model 2,† N = 482; 85 cases of anemia	
	aOR (95% CI)	P	aOR (95% CI)	P
Malaria	—	< 0.001	—	< 0.001
Yes	11.24 (4.98–25.37)	—	9.40 (4.08–21.66)	—
No	1	—	1	—
Iron deficiency—sTfR	—	—	—	0.004
Yes	—	—	2.70 (1.38–5.27)	—
No	—	—	1	—
Serum albumin (g/L)	—	0.004	—	0.004
< 35	2.14 (1.27–3.59)	—	2.18 (1.29–3.68)	—
≥ 35	1	—	1	—
C-reactive protein (mg/L)	—	0.022	—	0.026
≤ 5	1	—	1	—
> 5	1.94 (1.10–3.45)	—	1.93 (1.08–3.44)	—

aOR = adjusted odds ratio; sTfR = soluble transferrin receptor. Following variables have been removed by the stepwise backward model: mid upper arm circumference, level of education, number of pregnancy, age, α1-acid glycoprotein, retinol binding protein, fever during pregnancy, use of impregnated bed net, antimalarial treatment, iron deficiency defined by adjusted ferritin, deworming. In a second model using soluble transferrin receptor to define iron deficiency, the model did not remove this variable.

* Iron deficiency defined by adjusted ferritin.

† Iron deficiency defined by sTfR.

our study. Unfortunately, falciparum, the most virulent species, accounted for 95% of all malaria cases in our sample. The adjusted odds ratios were 11.24 (95% CI, 4.98–25.37) and 9.34 (4.08–21.66), respectively, when using SF and sTfR to evaluate iron load. These findings agree with previously published data.^{13,51–54} Malaria prevalence might have been underestimated in our study as we did use the microscopy to diagnose malaria. As stated above, submicroscopic malaria is common during pregnancy. Molecular techniques could detect more *Plasmodium* infection among pregnant women, which could very probably increase the proportion of anemia associated with malaria in our population. Malaria is known to induce anemia among pregnant women. Anemia during malaria infection may be mediated by many pathways, leading to either increased destruction or decreased production of RBC.⁵³

More than a fifth of pregnant women in our sample had raised CRP (22.5%) whereas AGP was raised in 7%. In combination (raised CRP and/or raised AGP) to define “inflammation,” the prevalence was found to be 23.9%; implying that CRP accounts for almost all the cases of inflammation. The positive association between inflammation and anemia found in our study is in accordance with previously published data.⁵⁵ The relationship between inflammation and anemia is not clearly understood. Inflammation induces anemia through complex pathological mechanisms involving, among others, inflammatory cytokines (interleukin-1, tumor necrosis factor- α , and interferon- γ).^{56,57} In response to inflammatory stimulus, hepcidin (a liver peptide) suppresses the expression of ferroportin (the iron export membrane protein) and so blocks iron efflux from macrophages (that leads to iron sequestration in reticuloendothelial cells) and also reduces iron absorption by duodenal enterocytes.^{56,58,59} During inflammation, anemia may also be induced by erythropoiesis inhibition⁵⁹ or by direct hemolysis or shortening of RBC survival.^{57,60} Impairment of erythropoietin production and blunted responsiveness of the bone marrow to erythropoietin have also been reported.⁵⁷

CONCLUSION

Our results have demonstrated that anemia is prevalent in pregnant women from the Miti-Murhesa health zone and is highly associated with malaria, inflammation, and undernutrition including low serum albumin. ID (evaluated by SF) is less prevalent and has not been found to be associated with anemia in our study population. Effective control of anemia during pregnancy in this region should consider fighting malaria and other infectious diseases in combination with measures to improve women's nutrition, both before and during pregnancy. It also exposes the lower than expected contribution of ID as a cause of anemia and therefore need for further investigation for other causes of anemia in pregnant women. The study further uncovers the need for more accurate indicators of iron load especially in a setting of high prevalence of infection/inflammation and hemoglobinopathies. For the current health policy in DRC, these findings should be taken into account. We suggest that such studies (including screening for hemoglobinopathies and genetic disorders) be carried out in all provinces because results might be different. But according to results from South Kivu province, health policy against anemia should be reevaluated, and some changes could be considered, at least moving to less

concentrated iron tablets for supplementation during pregnancy as suggested by WHO in its 2016 recommendations on ANC.⁶¹

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