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Incidence of iron deficiency anaemia in pregnant women after microcytic hypochromic anaemia: Prospective matched cohort study in Kisangani, Democratic Republic of the Congo

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ABSTRACT

Introduction

Iron deficiency anaemia (IDA) in pregnant women is a major public health challenge. The incidence of IDA increases throughout pregnancy, and women who experience it are at a similar risk of recurrence in subsequent pregnancies or even during the same pregnancy.

Purpose

This study aimed to determine the incidence of iron deficiency anaemia in pregnant women with microcytic hypochromic anaemia compared with pregnant women with non-microcytic hypochromic anaemia.

Methods

A prospective, open-label, multicentre, matched cohort study was conducted with 11,226 pregnant women, comprising 7,832 (69.77%) with microcytic hypochromic anaemia and 3,394 (30.23%) with non-microcytic hypochromic anaemia. These women were enrolled and monitored for 66 days in antenatal clinics (ANC) across 17 selected health facilities (HF) in Kisangani.

Results

The overall incidence of iron deficiency anaemia was 2.52% (95% CI: 2.47, 2.57) among all pregnant women. Women with microcytic hypochromic anaemia had a higher risk of developing iron deficiency anaemia compared to those with non-microcytic hypochromic anaemia (2.79% vs. 1.91%). Predictors of iron deficiency anaemia in pregnancy included microcytic hypochromic anaemia (aHR = 1.33; 95% CI: 1.26–1.40), age between 20 and 35 years (aHR = 1.19; 95% CI: 1.07–1.34), non-use of insecticide-treated nets (ITNs) (aHR = 1.11; 95% CI: 1.04–1.19), and iron supplementation during pregnancy (aHR = 1.14; 95% CI: 1.06–1.21).

Conclusion

The findings highlight the importance of preventing the progression of anaemia in pregnancy through improved reproductive health services.

INTRODUCTION

Iron deficiency anaemia (IDA) is a global public health concern, affecting survival and influenced by multiple factors. Understanding its epidemiology is essential for developing targeted interventions to improve health outcomes (Manish, 2025). IDA is a severe iron deficiency that impairs haem synthesis and reduces erythropoiesis (Kulik-Rechberger, 2024). It is the most widespread nutritional deficiency worldwide, affecting approximately 28.4% of the global population (Kumar et al., 2022).

In the Democratic Republic of the Congo (DRC), little information exists on IDA and its contribution to overall anaemia prevalence. Large-scale surveys such as the Demographic and Health Survey (DHS) or the Multiple Indicator Cluster Survey (MICS) have not assessed iron parameters, possibly due to the complexity of logistics and laboratory analyses required (Mbunga et al., 2021). IDA during pregnancy typically occurs in the later stages, even when iron stores are relatively adequate (World Health Organization [WHO], 2020), and affects about 50% of pregnant women in developing countries (WHO, 2015).

IDA in pregnancy is a major public health challenge because its incidence tends to increase throughout gestation (Ataide et al., 2023a), and women who experience it remain at a similar risk of recurrence in subsequent pregnancies (A. Kumar et al., 2022). A precursor to IDA, microcytic hypochromic anaemia is characterised by circulating red blood cells that are smaller than average (microcytic) and have reduced haemoglobin content, resulting in a pale appearance (hypochromic) (Shahzad et al., 2022). Microcytosis results from defective haemoglobin synthesis, reducing the mean corpuscular volume (MCV) to below 80 fL (Carlos et al., 2018). Hypochromia refers to a reduced ratio of haemoglobin to haematocrit, regardless of anaemia status (Choy et al., 2023). The predictive role of microcytosis and hypochromia in the development of IDA in pregnant women is well documented (Mohammed et al., 2020).

Several studies have identified key factors contributing to maternal IDA. Tamundele et al. (2023) demonstrated that iron supplementation, deworming, and antenatal counselling are effective in preventing IDA. Ataide et al. (2023b) reported that menstrual blood loss before conception contributes to iron depletion risk. Cubello et al. (2023) highlighted that maternal IDA combined with environmental lead exposure can adversely affect a child's neurological development. Davidson et al. (2023) found that pregnant women in resource-limited settings face high anaemia risks, but the aetiology of postpartum anaemia remains unclear.

Babah et al. (2024) confirmed that IDA is the primary cause of anaemia in pregnancy and that oral iron supplementation is widely recommended. Mean (2020) estimated that pregnancy consumes between 500 and 800 mg of iron in the mother, and that premenopausal women—particularly in less developed countries—often have marginal iron reserves or frank IDA. Mean also noted that pregnancy is associated with "physiological" anaemia due to increased maternal blood volume but is paradoxically accompanied by increased erythrocyte production and mass per kilogram of body weight.

Some statistical models assessing IDA have used inappropriate regression methods or failed to account for recurrent events, which may overestimate incidence rates (Schuster et al., 2020). Since no cohort study has thoroughly examined the relationship between microcytic hypochromic anaemia and IDA during pregnancy in the Congolese population, we conducted a matched cohort study in Kisangani, DRC, to determine the incidence of IDA in pregnant women with microcytic hypochromic anaemia compared with those with non-hypochromic, non-microcytic anaemia.

We hypothesised that the incidence of IDA is higher in pregnant women with microcytic hypochromic anaemia who are not receiving iron supplementation compared with those who are supplemented.

METHODS

Study Design

This prospective, matched, open-label, multicentre cohort study was conducted in Kisangani from 1 October 2022 to 19 June 2023. A pre-survey, conducted from 13 to 29 August 2022, preceded the study and involved 10% of the total health facility sample size to test the consistency of July 2022 antenatal care (ANC) and delivery data.

A collaborative framework was established between the Ministry of Health, the NGO Ema-Esu, and the principal

investigator. This agreement facilitated the purchase of laboratory equipment and supplies, as well as the rental of automated equipment from the Ema-Esu Laboratory, to ensure the reliability and validity of results in line with international standards.

Population

The study population comprised pregnant women enrolled in ANC at selected health facilities in Kisangani, diagnosed with microcytic hypochromic anaemia, and followed from the time of ANC enrolment, regardless of calendar date.

Sampling

Of the 46 health facilities providing quality maternal and child health care (American Academy of Pediatrics, 2017) and follow-up to delivery (World Health Organization [WHO], 2016), 13 were randomly selected. Four general referral hospitals (HGR) were added due to their capacity to provide refocused ANC and referral delivery services (WHO, 2018a), giving a total of 17 health facilities involved in the study.

Pregnant women with multiple pregnancies, a history of immune or haematological disorders, or blood transfusion in the past six months were excluded.

The sample size was determined using the "power logrank" command in Stata BE 17.0, based on the Freedman method with the option *nratio*(2) across the entire time spectrum, accounting for censoring (Machin et al., 2011). Following the example of unbalanced sampling (1:2) by Liu et al. (2013) and considering a 10% dropout rate, a withdrawal probability (*wdprob*) of 0.1, an α error of 0.05, and continuity correction, 456 events (failures) were required. This calculation yielded a total of 951 pregnant women—317 with microcytic hypochromic anaemia and 634 with non-microcytic hypochromic anaemia (Zuur, 2010).

Follow-Up of Iron Deficiency Anaemia Outcomes

Follow-up was administratively censored from study entry until the onset of IDA. The study period was divided into three intervals:

1. 1 October to 31 December 2022
2. 1 January to 31 March 2023
3. 1 April to 18 June 2023

Given that each pregnant woman should have 66 days of ANC follow-up (WHO, 2018b), inclusion began at the 14th week of amenorrhoea (SA) session. The study start date corresponded to the initial ANC visit, in line with WHO recommendations for positive pregnancy experiences (WHO, 2018a). The end date was defined as either the conclusion of the pregnancy episode or the completion of follow-up (Amorim, 2015).

The ANC sessions at the beginning and end of the study were designed to capture the full history of recurrent IDA events (Janvin et al., 2024; Bieszk-Stolorz, 2020) in the context of multiple failure studies, where participants may experience no event or multiple events (Castañeda & Gerritse, 2010; Westbury et al., 2016). From the 951 participants, a total of 1,902 potential observations were possible; however, only 1,871 were recorded.

Matching was performed using a 1:5 case–control format (Iwagami & Shinozaki, 2022). Controls were matched on variables likely to explain survival differences (Thomas et al., 2020). Risk incidence was used as the matching estimator since not all women with eligible IDA diagnoses necessarily had microcytic hypochromic anaemia (Mostafa et al., 2022). The matching process resulted in 9,355 control observations, yielding a final cohort of 11,226 pregnant women.

Instruments, Data Collection, and Data Linkage

Venous blood samples were collected from each participant at the health facility and analysed at the Ema-Esu Reference Laboratory in Kisangani. Complete blood count (CBC) results included anaemia (Hb < 10.5 g/dL), normocytosis (MCV: 82–98 fL), microcytosis (MCV < 82 fL), macrocytosis (MCV > 98 fL), normochromia (MCHC: 316–360 g/L), and hypochromia (MCHC < 316 g/L) (Doig & Zhang, 2017). The MCV–MCHC–RDW combination model of Tong et al. (2017) was used to diagnose IDA, with 94% sensitivity and 90% specificity.

Plasma ferritin levels were measured to assess iron stores (Pfeiffer & Looker, 2017) using the AssayMax Human Ferritin ELISA kit (AssayPro, USA) (Bleicher et al., 2018), with an intra-assay coefficient of variation of 2.9% and calibration against WHO standards (Loy et al., 2019). Ferritin thresholds classified participants as severely iron-depleted (<15 µg/L), moderately iron-depleted (15–<30

µg/L), or iron-sufficient (≥30 µg/L) (Garzon et al., 2020; Pavord et al., 2020).

Sociodemographic, clinical, and nutritional data were extracted from ANC records and the national health information system (SNIS) database and linked with laboratory results.

Exposure and Outcome Variables

The primary exposure was microcytic hypochromic anaemia, recognised as a predictor of IDA (Mohammed et al., 2020). The primary outcome was the time to occurrence of maternal IDA. Cases of hypochromic–microcytic anaemia with moderate or severe iron depletion were considered events, whereas non-hypochromic, non-microcytic cases and iron-sufficient participants were censored.

Although moderate iron depletion does not entirely prevent progression to severe depletion, it was hypothesised that plasma hepcidin concentrations would vary according to iron loading and erythropoietic stimulation during pregnancy (Wang, 2016). Hepcidin deficiency leads to iron overload in haemochromatosis and anaemias with ineffective erythropoiesis, whereas excess hepcidin causes iron-restrictive anaemias, including anaemia of inflammation (Nemeth & Ganz, 2023).

Control Variables

Potential prognostic factors for recurrent IDA were identified from the literature. Loy et al. (2019) associated age <35 years, low education, multiparity, and lack of iron supplementation with increased risk. Breymann (2013) identified high-risk groups as women in later pregnancy, those with pre-existing IDA, multiple gestations, short interpregnancy intervals, multiparity, and low socioeconomic status.

Additional variables included maternal age (<20, 20–35, 36–46 years) (Biete et al., 2023), BMI at pregnancy onset and end (<18.5, 18.5–24.0, 24.0–28.0 kg/m²) (Xie et al., 2021), use of insecticide-treated nets (ITNs) (Ansah et al., 2024), intermittent preventive treatment with sulfadoxine–pyrimethamine (Moukoko et al., 2023), mebendazole use (Maternal Health Division Ministry of Health and Family Welfare, 2014), and folic acid plus iron supplementation (Finkelstein et al., 2024). Parity categories were primiparous (one delivery), multiparous (≥2 deliveries), and nulliparous (zero deliveries) (Chloe et al., 2014). Laboratory-confirmed intestinal worm infection (WHO, 2012) and malaria occurrence (Unger et al., 2023) were also recorded.

Data Quality Assurance

Data quality was ensured through careful design and pre-testing of data collection tools, training of data collectors and supervisors, daily review of forms by the principal investigator, and prompt correction of inconsistencies.

Statistical Analysis

Categorical covariates were harmonised, and frequencies with percentages were calculated. A chi-square (χ^2) test examined the association between IDA status and categorical variables.

The incidence rate (IR) of IDA and its 95% confidence interval (CI) were estimated using Poisson regression, expressed per 100 person-years. Stratified multivariate Cox regression models examined the association between microcytic hypochromic anaemia and IDA risk, allowing for separate baselines for each matched set (Johansson, 2024). Effect sizes were expressed as hazard ratios (HR) with 95% CIs. All analyses were conducted using Stata/BE 17.0 (StataCorp, TX, USA).

RESULTS

General characteristics

The basic characteristics of the study participants are presented in **Table 1**.

A total of 11,226 pregnant women were included in the analysis: 7,851 (69.94%) with microcytic hypochromic anaemia and 3,375 (30.06%) with non-microcytic hypochromic anaemia. Anaemia during pregnancy is a major concern regardless of type. Microcytic hypochromic anaemia strongly suggests iron deficiency anaemia (IDA), the most common nutritional deficiency worldwide, particularly among pregnant women. Non-microcytic hypochromic anaemia may indicate other types of anaemia, such as anaemia of chronic disease or early stages of other deficiencies (Obeagu, 2024).

Overall, the results were statistically significant. Most participants were aged 20–35 years (73.13%), married (70.77%), had a normal weight both at the beginning and

end of pregnancy (50.44% vs. 43.06%), and were educated (81.61%). Many pregnant women did not use insecticide-treated nets (ITNs) (71.04%) and were not supplemented with iron (67.44%), sulfadoxine-pyrimethamine (SP) (70.58%), or mebendazole (75.50%). More than half had intestinal worms detected in stool tests (59.92%) and had developed malaria during pregnancy (52.34%). Health facilities in the Makiso-Kisangani health zone registered the highest proportion of participants (52.71%), while the Tshopo health zone recorded only 5.71%.

Marital status is an important social determinant of health, influencing healthcare utilisation and outcomes, but it is not a direct indicator of clinical disease. It affects access to resources and support systems, which in turn impact health (Pandey et al., 2019). The observed decrease in the percentage of women with normal weight during pregnancy (from 50.44% to 43.06%), while not alarming on its own, may indicate inadequate gestational weight gain or even weight loss in some individuals—both of which can pose risks to fetal growth. Without further context, such as pre-pregnancy BMI categories and average weight gain, it is difficult to draw definitive conclusions about the significance of this change (Tingyuan Wen, 2015).

Higher education levels are generally associated with better health literacy and health-seeking behaviours (Afşar & Özkan, 2023). In malaria-endemic areas, not using ITNs significantly increases the risk of infection. ITNs are a cost-effective prevention method, reducing malaria transmission by preventing mosquito bites (Kuetche et al., 2023). Iron supplements, SP, and mebendazole are crucial preventive interventions during pregnancy: iron addresses IDA, SP (as part of intermittent preventive treatment in pregnancy) prevents malaria, and mebendazole prevents anaemia and nutrient malabsorption caused by intestinal worms (WHO, 2016).

Intestinal worms, particularly hookworm, cause chronic blood loss and malabsorption, leading to IDA. Malaria—especially *Plasmodium falciparum* infection—can cause haemolytic anaemia, fever, and adverse pregnancy outcomes such as miscarriage, preterm birth, and low birth weight. Co-infection can worsen anaemia and increase the risk of poor birth outcomes (Tamir et al., 2025).

The difference in patient registration between the Makiso-Kisangani (52.71%) and Tshopo (5.71%) health zones indicates a substantial disparity in healthcare utilisation or reporting. Low registration in Tshopo may reflect reduced access to facilities, low utilisation even when access exists, or inadequate data collection (Longchar et al., 2025).

The χ^2 test revealed significant differences in the occurrence of iron deficiency anaemia. Microcytic hypochromic anaemia, age, body mass index at both the beginning and end of pregnancy, parity, gestation, ITN use, iron, SP, and mebendazole supplementation, intestinal worm infection, malaria diagnosis, and health zone of consultation were all highly significantly associated with IDA risk ($p < 0.001$). Marital status and education were also significantly associated ($p = 0.004$ and $p = 0.007$, respectively).

Incidence of Maternal Iron-Deficiency Anemia

The overall incidence of iron-deficiency anemia was 2.54% (95% CI: 2.49–2.59). The risk of developing iron-deficiency anemia was 2.81% for pregnant women with microcytic hypochromic anemia compared to 1.94% for those with non-microcytic hypochromic anemia. **Table 2** shows the incidence rates of iron-deficiency anemia in pregnant women.

The incidence of iron-deficiency anemia in pregnant women aged 36–49 was 2.65% (95% CI: 2.45–2.86). The risk of developing iron-deficiency anemia was 2.74% (95% CI: 2.51–3.00) in pregnant women aged 36 to 49 with microcytic hypochromic anemia, compared to 2.38% (95% CI: 2.03–2.80) in those without microcytic hypochromic anemia.

The incidence of iron-deficiency anemia in underweight pregnant women at the beginning and end of pregnancy was 2.63% (95% CI: 2.53–2.74) and 2.66% (95% CI: 2.55–2.77), respectively. The risk of developing iron-deficiency anemia in pregnant women with microcytic hypochromic anemia was 2.86% (95% CI: 2.73–2.99) at the start and 2.86% (95% CI: 2.74–3.00) at the end of pregnancy, compared with 2.05% (95% CI: 1.88–2.24) and 2.08% (95% CI: 1.90–2.27) for those without microcytic hypochromic anemia.

Similarly, the incidence of iron-deficiency anemia in multiparous pregnant women was 2.57% (95% CI: 2.49–2.66). The risk was 2.78% (95% CI: 2.75–2.96) for

multiparous women with microcytic hypochromic anemia, versus 1.80% (95% CI: 1.66–1.94) for those without.

The incidence among multigestational women was 2.54% (95% CI: 2.48–2.61). The risk of developing iron-deficiency anemia was 2.82% (95% CI: 2.74–2.90) for those with microcytic hypochromic anemia, compared to 1.96% (95% CI: 1.87–2.06) for those without.

Among married pregnant women, the incidence was 2.56% (95% CI: 2.50–2.62), with risks of 2.78% (95% CI: 2.70–2.85) for those with microcytic hypochromic anemia and 2.04% (95% CI: 1.94–2.14) for those without.

Pregnant women attending school had an incidence of 2.55% (95% CI: 2.49–2.60). The risk was 2.83% (95% CI: 2.76–2.90) for those with microcytic hypochromic anemia, compared to 1.93% (95% CI: 1.85–2.02) for those without.

The incidence in pregnant women not using insecticide-treated nets (ITNs) was 2.62% (95% CI: 2.56–2.68). The risk was 2.85% (95% CI: 2.78–2.93) for those with microcytic hypochromic anemia, compared to 1.98% (95% CI: 1.88–2.08) for those without.

The incidence of iron-deficiency anemia in pregnant women not supplemented with sulfadoxine-pyrimethamine (SP), iron, and mebendazole was 2.62% (95% CI: 2.56–2.68), 2.80% (95% CI: 2.74–2.87), and 2.56% (95% CI: 2.56–2.68), respectively. Corresponding risks for those with microcytic hypochromic anemia were 2.84% (95% CI: 2.76–2.91), 2.89% (95% CI: 2.82–2.96), and 2.82% (95% CI: 2.75–2.89); for those without, risks were 1.98% (95% CI: 1.88–2.09), 1.98% (95% CI: 1.88–2.09), and 2.07% (95% CI: 1.97–2.17), respectively.

Pregnant women with intestinal worms in the stool had an incidence of 2.77% (95% CI: 2.70–2.84). The risk was 2.87% (95% CI: 2.80–2.95) for those with microcytic hypochromic anemia, versus 2.22% (95% CI: 2.08–2.38) for those without. The incidence in pregnant women with malaria was 2.68% (95% CI: 2.57–2.75), with risks of 2.86% (95% CI: 2.78–2.94) for those with microcytic hypochromic anemia and 2.06% (95% CI: 1.94–2.20) for those without.

Among pregnant women attending health facilities in the Tshopo health zone, the incidence was 2.63% (95% CI: 2.43–2.85). The risk was 2.70% (95% CI: 2.47–2.96) for those with microcytic hypochromic anemia and 2.41% (95% CI: 1.78–2.86) for those without.

This provides a reference point for iron-deficiency anemia in pregnant women: approximately 2 to 3 out of every 100 pregnant women will develop it. Although this percentage may seem low, it can represent a significant number of affected individuals in a large population, constituting a major public health concern. The narrow 95% confidence interval (CI: 2.49–2.59) indicates a precise estimate, suggesting that the result is robust and unlikely to be due to chance (Obianeli et al., 2024).

Microcytic hypochromic anemia — characterised by small, pale red blood cells — is typical of iron deficiency, since iron is essential for haemoglobin production, which gives red blood cells their colour and size (Li et al., 2022a). Thus, pregnant women with microcytic hypochromic anemia have a significantly higher likelihood of iron deficiency (2.81% versus 1.94% in those without).

Pregnant women diagnosed with microcytic hypochromic anemia should be evaluated further for iron deficiency and, if confirmed, promptly given iron supplementation. This can prevent progression of iron-deficiency anemia and its complications for both mother and fetus (Mean, 2020).

Identifying high-risk groups such as pregnant women allows healthcare providers to allocate resources like blood tests, supplements, and dietary advice more strategically, leading to better treatment and improved outcomes. This targeted approach ensures that those most in need receive appropriate support to prevent or address iron deficiency (Ge et al., 2025).

Table 1:
Baseline characteristics of pregnant women exposed and unexposed to microcytic hypochromic anemia and diagnosed as iron-deficiency anemia

		Microcytic hypochromic anemia		**Iron deficiency anemia** (IDA)		
Characteristic	**Total Patients**	**Present**	**Absence**	**IDA Severe**	**IDA Moderate**	**Sufficiency iron**
Patients N (%)	11,226	7,851 (69.94)	3,375 (30.06)	6,297 (56.09)	3,660 (32.60)	1,269 (11.30)
MHA*						$p < 0.001$
No	3,375 (30.06)			386 (6.13)	1,950 (53.28)	1,039 (81.88)
Yes	7,851 (69.94)			5,911 (93.87)	1,710 (46.72)	230 (18.12)
Age (years)			$p < 0.001$			$p < 0.001$
12-19	2,347 (20.91)	1,688 (21.50)	659 (19.53)	1,282 (20.36)	712 (19.45)	353 (27.82)
20-35	8,210 (73.13)	5,662 (72.12)	2,548 (75.50)	4,627 (73.48)	2,707 (73.96)	876 (69.03)
36-49	669 (5.96)	501 (6.38)	168 (4.98)	388 (6.16)	241 (6.58)	40 (3.15)
BMI start			$p < 0.001$			$p < 0.001$
Normal weight	5,626 (50.44)	4,009 (51.06)	1,653 (48.98)	3,229 (51.28)	1,740 (47.54)	693 (54.61)
Underweight	2,600 (23.16)	1,897 (24.16)	703 (20.83)	1,618 (25.69)	748 (20.44)	234 (18.44)
Overweight	2,964 (26.40)	1,945 (24.77)	1,019 (30.19)	1,450 (23.03)	1,172 (32.02)	342 (26.95)
BMI end			$p < 0.001$			$p < 0.001$
Normal weight	4,834 (43.06)	3,363 (42.84)	1,471 (43.59)	2,708 (43.00)	1,510 (41.26)	616 (48.54)
Underweight	2,564 (22.84)	1,925 (24.52)	639 (18.93)	1,639 (26.03)	714 (19.51)	211 (16.63)
Overweight	3,828 (34.10)	2,563 (32.64)	1,265 (37.48)	1,950 (30.97)	1,436 (39.23)	442 (34.83)
Parity			$p < 0.001$			$p < 0.001$
Nulliparous	3,936 (35.06)	2,839 (36.16)	1,097 (32.59)	2,244 (35.64)	1,263 (34.51)	429 (33.81)
Primipare	3,463 (30.85)	2,147 (27.35)	1,316 (38.99)	1,733 (27.52)	1,303 (35.60)	427 (33.65)
Multipare	3,827 (34.09)	2,865 (36.49)	962 (28.50)	2,320 (36.84)	1,094 (29.89)	413 (32.55)
Gestation			$p < 0.001$			$p = 0.680$
Primigeste	4,042 (36.01)	2,920 (37.19)	1,122 (33.24)	2,288 (36.33)	1,307 (35.71)	447 (35.22)
Multigeste	7,184 (63.99)	4,931 (62.81)	2,253 (66.76)	4,009 (63.67)	2,353 (64.29)	822 (64.78)
Marital status			$p = 0.004$			$p = 0.016$
Single	3,281 (29.23)	2,231 (28.42)	1,050 (31.11)	1,802 (28.62)	1,065 (29.10)	414 (32.62)
Married	7,945 (70.77)	5,620 (71.58)	2,325 (68.89)	4,495 (71.38)	2,595 (70.90)	855 (67.38)
Education			$p = 0.007$			$p = 0.064$
Educated	9,161 (81.61)	6,356 (80.96)	2,805 (83.11)	5,096 (80.93)	3,031 (82.81)	1,034 (81.48)
No educated	2,065 (18.39)	1,495 (19.04)	570 (16.89)	1,201 (19.07)	629 (17.19)	235 (18.52)
ITN* use			$p < 0.001$			$p < 0.001$
Use	3,251 (28.96)	1,957 (24.93)	1,294 (38.34)	1,323 (21.01)	1,415 (38.66)	513 (40.43)
Don't use	7,975 (71.04)	5,894 (75.07)	2,081 (61.66)	4,974 (78.99)	2,245 (61.34)	756 (59.57)
SP* intake			$p < 0.001$			$p < 0.001$
Yes	3,303 (29.42)	1,933 (24.62)	1,370 (40.59)	1,190 (18.90)	1,554 (42.46)	559 (44.05)
No	7,923 (70.58)	5,918 (75.38)	2,005 (59.41)	5,107 (81.10)	2,106 (57.54)	710 (55.95)
Iron intake			$p < 0.001$			$p < 0.001$
Yes	3,655 (32.56)	1,505 (19.17)	2,150 (63.70)	360 (5.72)	2,302 (62.90)	993 (78.25)
No	7,571 (67.44)	6,346 (80.83)	1,225 (36.30)	5,937 (94.28)	1,358 (37.10)	276 (21.75)
Mebendazole			$p < 0.001$			$p < 0.001$
Yes	2,750 (24.50)	1,627 (20.72)	1,123 (33.27)	979 (15.55)	1,235 (33.74)	536 (42.24)
No	8,476 (75.50)	6,224 (79.28)	2,252 (66.73)	5,318 (84.45)	2,425 (66.26)	733 (57.76)
Hookworms			$p < 0.001$			$p < 0.001$
No	4,499 (40.08)	2,201 (28.03)	2,298 (68.09)	892 (14.17)	2,664 (72.79)	943 (74.31)
Yes	6,727 (59.92)	5,650 (71.97)	1,077 (31.91)	5,405 (85.83)	996 (27.21)	326 (25.69)
Malaria			$p < 0.001$			$p < 0.001$
No	5,350 (47.66)	3,281 (41.79)	2,069 (61.30)	2,099 (33.33)	2,433 (66.48)	818 (64.46)
Yes	5,876 (52.34)	4,570 (58.21)	1,306 (38.70)	4,198 (66.67)	1,227 (33.52)	451 (35.54)
Patients N (%)	11,226	7,851 (69.94)	3,375 (30.06)	6,297 (56.09)	3,660 (32.60)	1,269 (11.30)
Healthcare			$p < 0.001$			$p < 0.001$
Kabondo	2,917 (25.98)	1,892 (24.10)	1,025 (30.37)	1,610 (25.57)	1,009 (27.57)	298 (23.48)
Makiso-Kisangani	5,917 (52.71)	4,254 (54.18)	1,663 (49.27)	3,470 (55.11)	1,821 (49.75)	626 (49.33)
Mangobo	1,751 (15.60)	1,248 (15.90)	503 (14.90)	874 (13.88)	588 (16.07)	289 (22.77)
Tshopo	641 (5.71)	457 (5.82)	184 (5.46)	343 (5.45)	242 (6.61)	56 (4.41)

Table 2:
Incidence Rates of Iron-Deficiency Anemia in Pregnant Women with Microcytic Hypochromic Anemia in the Full Cohort

	Number of events /Person-time			Incidence rate per 100 Person-Time (95% CI)		
Exposure	**Total**	**MHA***	**No MHA***	**Total**	**MHA***	**No MHA***
Patients	**9,957/391,493**	**7,621/271,250**	**2,336/120,243**	**2.54 (2.49, 2.59)**	**2.81 (2.75, 2.87)**	**1.94 (1.87, 2.02)**
Age, years						
12-19	1,994/82,886	1,600/58,782	394/23,707	2.42 (2.31, 2.53)	2.72 (2.59, 2.86)	1.66 (1.51, 1.83)
20-35	7,344/285,264	5,540/194,938	1,794/90,326	2.57 (2.51, 2.63)	2.84 (2.77, 2.92)	1.99 (1.90, 2.08)
36-49	629/23,740	481/17,530	148/6,210	2.65 (2.45, 2.86)	2.74 (2.51, 3.00)	2.38 (2.03, 2.80)
Patients	**9,957/391,493**	**7,621/271,250**	**2,336/120,243**	**2.54 (2.49, 2.59)**	**2.81 (2.75, 2.87)**	**1.94 (1.87, 2.02)**
BMI start						
Normal weight	4,969/197,909	3,871/139,591	1,098/58,318	2.51 (2.44, 2.58)	2.77 (2.69, 2.86)	1.88 (1.77, 2.00)
Underweight	2,366/89,982	1,847/64,693	519/25,289	2.63 (2.53, 2.74)	2.86 (2.73, 2.99)	2.05 (1.88, 2.24)
Overweight	2,622/103,602	1,903/66,966	719/36,636	2.53 (2.44, 2.63)	2.84 (2.72, 2.97)	1.96 (1.82, 2.11)
BMI end						
Normal weight	4,218/170,086	3,241/118,322	977/51,764	2.48 (2.41, 2.56)	2.74 (2.65, 2.84)	1.89 (1.77, 2.01)
Underweight	2,353/88,540	1,871/65,313	482/23,227	2.66 (2.55, 2.77)	2.86 (2.74, 3.00)	2.08 (1.90, 2.27)
Overweight	3,386/132,867	2,509/87,615	877/45,252	2.55 (2.46, 2.64)	2.85 (2.74, 2.98)	1.94 (1.81, 2.07)
Parity						
Nulliparous	3,507/137,892	2,755/99,146	752/38,746	2.54 (2.46, 2.63)	2.78 (2.68, 2.88)	1.94 (1.81, 2.08)
Primipare	3,036/120,855	2,089/74,804	947/46,051	2.51 (2.42, 2.60)	2.79 (2.68, 2.91)	2.06 (1.93, 2.19)
Multipare	3,414/132,746	2,777/97,300	637/35,446	2.57 (2.49, 2.66)	2.78 (2.75, 2.96)	1.80 (1.66, 1.94)
Gestation						
Primigeste	3,595/141,342	2,836/101,577	759/39,764	2.54 (2.46, 2.63)	2.79 (2.69, 2.90)	1.91 (1.78, 2.05)
Multigeste	6,362/250,152	4,785/169,673	1,577/80,479	2.54 (2.48, 2.61)	2.82 (2.74, 2.90)	1.96 (1.87, 2.06)
Marital status						
Single	2,867/114,427	2,203/76,147	664/38,280	2.51 (2.42, 2.60)	2.89 (2.77, 3.02)	1.73 (1.61, 1.87)
Married	7,090/277,066	5,418/195,103	1,72/81,963	2.56 (2.50, 2.62)	2.78 (2.70, 2.85)	2.04 (1.94, 2.14)
Education						
Educated	8,127/319,038	6,192/218,773	1,935/100,265	2.55 (2.49, 2.60)	2.83 (2.76, 2.90)	1.93 (1.85, 2.02)
No educated	1,830/72,455	1,429/52,477	401/19,978	2.53 (2.41, 2.64)	2.2 (2.59, 2.87)	2.01 (1.82, 2.21)
ITN* net						
Use	2,738/115,763	1,864/69,464	874/46,299	2.37 (2.28, 2.46)	2.68 (2.56, 2.81)	1.89 (1.77, 2.02)
Don't use	7,219/275,730	5,757/201,786	1,462/73,944	2.62 (2.56, 2.68)	2.85 (2.78, 2.93)	1.98 (1.88, 2.08)
SP* intake						
Yes	2,744/115,860	1,812/66,457	932/49,403	2.37 (2.28, 2.46)	2.73 (2.60, 2.86)	1.89 (1.77, 2.01)
No	7,213/275,633	5,809/204,793	1,404/70,840	2.62 (2.56, 2.68)	2.84 (2.76, 2.91)	1.98 (1.88, 2.09)
Iron intake						
Yes	2,662/131,094	1,311/53,058	1,351/78,036	2.03 (1.95, 2.11)	2.47 (2.34, 2.61)	1.73 (1.64, 1.83)
No	7,295/260,399	6,310/218,192	985/42,207	2.80 (2.74, 2.87)	2.89 (2.82, 2.96)	2.28 (2.19, 2.48))
Mebendazole						
Yes	2,214/95,632	1,538/55,607	676/40,025	2.32 (2.22, 2.41)	2.77 (2.63, 2.91)	1.69 (1.57, 1.82)
No	7,743/295,861	6,083/215,643	1,660/80,218	2.56 (2.56, 2.68)	2.82 (2.75, 2.89)	2.07 (1.97, 2.17)
Hookworms						
No	3,556/160,118	2,040/76,753	1,516/83,365	2.22 (2.15, 2.30)	2.66 (2.55, 2.78)	1.82 (1.73, 1.91)
Yes	6,401/231,375	5,581/194,497	820/36,878	2.77 (2.70, 2.84)	2.87 (2.80, 2.95)	2.22 (2.08, 2.38)
Malaria						
No	4,532/188,995	3,137/114,344	1,395/74,651	2.40 (2.33, 2.47)	2.74 (2.65, 2.84)	1.87 (1.77, 1.97)
Yes	5,425/202,498	4,484/156,906	941/45,592	2.68 (2.57, 2.75)	2.86 (2.78, 2.94)	2.06 (1.94, 2.20)
Healthcare						
Kabondo	2,619/101,280	1,824/63,073	795/38,207	2.59 (2.49, 2.69)	2.89 (2.76, 3.03)	2.08 (1.94, 2.23)
Makiso-Kisangani	5,291/206,350	4,146/146,998	1,145/59,352	2.56 (2.50, 2.63)	2.82 (2.74, 2.91)	1.93 (1.79, 2.04)
Mangobo	1,462/61,640	1,194/44,276	268/17,364	2.37 (2.25, 2.50)	2.70 (2.55, 2.85)	1.54 (1.37, 1.74)
Tshopo	585/22,223	457/16,903	128/5,320	2.63 (2.43, 2.85)	2.70 (2.47, 2.96)	2.41 (1.78, 2.86)

Abbreviations: MHA = Microcytic hypochromic anemia; ITN* = Insecticide-treated nets; SP* = Sulphadoxine-pyrimethamine*

Factors Predicting the Incidence of Iron-Deficiency Anemia in Pregnancy

Multivariate Cox stratified regression analysis showed that microcytic hypochromic anemia, mean age between 20 and 35 years, being underweight at the start and end of pregnancy, marriage, lack of use of insecticide-treated mosquito nets, iron supplementation, and the location of health centres were predictive factors for the development of iron-deficiency anemia in pregnant women.

Table 3 reports the detailed analysis of the risk of iron-deficiency anemia during pregnancy using the Cox stratified proportional hazards model.

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Table 3:
Stratified Cox Proportional Hazards Model Analysis on the Risk of Iron-Deficiency Anemia in Pregnancy

	Total		*Microcytic hypochromic anemia*		*No Microcytic hypochromic anemia*	
Exposure	**Adjusted HR**	***P***	**Adjusted HR**	***P***	**Adjusted HR**	***P***
MHA*						
Yes	1.33 (1.26, 1.40)	**0.000**				
Age, years						
20-35	1.06 (1.00, 1.11)	**0.025**	1.03 (0.97, 1.09)	0.328	1.19 (1.07, 1.34)	**0.002**
36-49	1.04 (0.95, 1.15)	0.309	0.97 (0.87, 1.07)	0.582	1.15 (0.94, 1.40)	0.159
BMI start						
Underweight	0.86 (0.76, 0.96)	**0.013**	0.80 (0.70, 0.91)	**0.001**	1.21 (0.93, 1.57)	0.145
Overweight	1.01 (0.94, 1.10)	0.661	0.99 (0.90, 1.08)	0.839	1.06 (0.89, 1.26)	0.055
BMI end						
Underweight	1.19 (1.06, 1.34)	**0.003**	1.27 (1.12, 1.45)	**0.000**	0.87 (0.66, 1.14)	0.320
Overweight	1.07 (0.99, 1.15)	0.068	1.10 (1.01, 1.20)	**0.018**	0.96 (0.81, 1.13)	0.657
Parity						
Primipare	1.08 (0.95, 1.22)	0.234	1.14 (0.99, 1.30)	0.055	0.32 (0.15, 0.69)	**0.004**
Multipare	1.07 (0.94, 1.23)	0.274	1.22 (1.05, 1.41)	0.006	0.26 (0.12, 0.57)	**0.001**
Gestation						
Multigeste	0.96 (0.85, 1.10)	0.641	0.91 (0.79, 1.04)	0.197	3.18 (1.50, 6.74)	**0.002**
Marital status						
Married	0.92 (0.88, 0.97)	**0.001**	0.84 (0.80, 0.89)	**0.000**	1.15 (1.04, 1.28)	**0.004**
Education						
No educated	0.96 (0.91, 1.02)	0.229	0.94 (0.88, 1.00)	0.065	0.94 (0.84, 1.06)	0.367
ITN use						
Don't use	1.07 (1.02, 1.13)	**0.005**	1.11 (1.04, 1.19)	**0.001**	0.97 (0.88, 1.08)	0.679
SP intake						
No	0.97 (0.92, 1.03)	0.437	0.93 (0.87, 0.99)	**0.046**	1.03 (0.93, 1.15)	0.507
Iron intake						
No	1.21 (1.14, 1.28)	**0.000**	1.14 (1.06, 1.21)	**0.000**	1.34 (1.21, 1.48)	**0.000**
Mebendazole						
No	0.95 (0.90, 1.00)	0.061	0.91 (0.86, 0.97)	**0.004**	1.01 (0.92, 1.12)	0.727
Hookworms						
Yes	1.05 (0.99, 1.13)	0.085	1.03 (0.95, 1.11)	0.431	1.17 (1.01, 1.35)	**0.029**
Malaria						
Yes	0.98 (0.92, 1.04)	0.609	1.00 (0.93, 1.07)	0.957	0.91 (0.77, 1.06)	0.242
Healthcare						
Makiso-	0.93 (0.89, 0.98)	**0.013**	0.94 (0.88, 1.00)	0.055	0.88 (0.80, 0.98)	**0.024**
Kisangani	0.80 (0.75, 0.86)	**0.000**	0.84 (0.77, 0.91)	**0.000**	0.67 (0.57, 0.78)	**0.000**
Mangobo	0.95 (0.87, 1.04)	0.351	0.88 (0.79, 0.98)	**0.024**	1.35 (1.10, 1.65)	**0.004**
Tshopo						

*HR: Hazard Ratio. MHA: Microcytic hypochromic anemia. ITN: Insecticide-treated nets. SP: Sulphadoxine-pyrimethamine.

Pregnant women with microcytic hypochromic anemia had an overall 33% greater risk of developing iron-deficiency anemia than their counterparts without microcytic hypochromic anemia (aHR = 1.33; 95% CI: 1.26–1.40). Pregnant women aged between 20 and 35 had an overall 6% higher risk of developing iron-deficiency anemia than those aged 12 to 19 (aHR = 1.06; 95% CI: 1.00–1.11), over 19% of whom had microcytic hypochromic anemia (aHR = 1.19; 95% CI: 1.07–1.34).

Pregnant women who were underweight both at the beginning and end of pregnancy had an overall risk of 14% lower and over 19% higher, respectively, of developing iron-deficiency anemia compared with their normal-weight counterparts (aHR = 0.86; 95% CI: 0.76–0.96 and aHR = 1.19; 95% CI: 1.06–1.34). Among them, less than 20% and over 27% had microcytic hypochromic anemia (aHR = 0.80; 95% CI: 0.70–0.91 and aHR = 1.27; 95% CI: 1.12–1.45), respectively.

Married pregnant women had an overall 8% lower risk of developing iron-deficiency anemia than unmarried women (aHR = 0.92; 95% CI: 0.88–0.97), with less than 16% having microcytic hypochromic anemia (aHR = 0.84; 95% CI: 0.80–0.89).6

Pregnant women who did not use ITNs during pregnancy had an overall 7% greater risk of developing iron-deficiency anemia than those who used ITNs (aHR = 1.07; 95% CI: 1.02–1.13), with over 11% having microcytic hypochromic

anemia (aHR = 1.11; 95% CI: 1.04–1.19). Pregnant women who were not supplemented with iron during pregnancy had an overall 21% higher risk of developing iron-deficiency anemia than their supplemented counterparts (aHR = 1.21; 95% CI: 1.14–1.28), with over 14% having microcytic hypochromic anemia (aHR = 1.14; 95% CI: 1.06–1.21).

Pregnant women attending health facilities in the Makiso-Kisangani and Mangobo health zones had an overall 7% and 20% lower risk of developing iron-deficiency anemia, respectively, compared to those in Kabondo (aHR = 0.93; 95% CI: 0.89–0.98 and aHR = 0.80; 95% CI: 0.75–0.94), with less than 16% developing microcytic hypochromic anemia in the Mangobo health zone (aHR = 0.84; 95% CI: 0.77–0.91).

The bivariate analysis presented in **Table 2** indicates that pregnant women with microcytic hypochromic anemia had a higher risk of developing iron-deficiency anemia than those without (2.81% vs. 1.94%), consistent with the Cox proportional hazards model in **Table 3** (adjusted HR: 1.46; 95% CI: 1.39–1.53).

A cohort study, involving observation and follow-up over time with each event recorded, is suitable for studying the incidence and risk factors of maternal iron-deficiency anemia (CDC, 2022). In clinical research, cohort studies are appropriate when reasonable evidence exists of an association between exposure and outcome, including the time interval between exposure and outcome development (Wang & Kattan, 2020). Prospective collection of biomarker data avoids information bias in retrospective data and selection bias from faulty sampling of base populations (Brien et al., 2023). Since results of cohort studies are often attributed to confounding factors (Norgaard et al., 2017), matching reduces confounding and improves the likelihood that controls represent what cases would have been if unexposed (Howards, 2018). Matching case selection is transparent, reproducible, and protects research from criticism that cases were intentionally chosen to bias results (Nielsen, 2016).

Clinical analysis of the risk of iron-deficiency anemia during pregnancy using the Cox proportional hazards model involves studying a cohort of pregnant women to identify factors influencing the time to development of anemia (Lee et al., 2023). This model assesses the effect of various risk factors on the hazard rate of anemia onset, accounting for the time until the event occurs (Deo & Deo, 2021).

In pregnant women, particularly in developing countries, most microcytic hypochromic anemia cases are attributed to iron deficiency, indicating it as the primary underlying cause when these red blood cell indices are observed (Kebede et al., 2025).

Iron-deficiency anemia (IDA) during pregnancy, especially when presented as microcytic hypochromic anemia, is associated with several adverse maternal and fetal outcomes. Maternal risks include preterm delivery (Benson et al., 2024), postpartum hemorrhage (Lao et al., 2022), and increased risk of cesarean section due to complications such as fetal distress. Fetal risks include low birth weight (Georgieff, 2023), intrauterine growth restriction (Obeagu, 2024), and potential neurodevelopmental delays (Obeagu et al., 2025). This underlines the need to:

- Implement effective prenatal screening for anemia, including red blood cell indices and iron stores such as ferritin, to identify and treat early deficiencies. This proactive approach helps prevent complications such as premature birth, low birth weight, and postpartum hemorrhage (Cantor et al., 2024).
- Promote proactive iron supplementation, whether universal or targeted to high-risk groups, due to the high prevalence and physiological importance of iron. This is particularly crucial in populations where anemia and iron deficiency are widespread, including infants, young children, pregnant women, and menstruating women (Coyne, 2022).
- Educate pregnant women about iron-rich foods and factors affecting iron absorption, significantly improving their knowledge, attitudes, and practices regarding iron intake (Verulava & Gogua, 2024).
- Address underlying socio-economic factors contributing to nutritional deficiencies, such as poverty, lack of education, and limited access to resources, which create barriers to healthy food choices and increase the risk of malnutrition (Brink et al., 2022).

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- Improve adherence to iron supplementation through multifaceted strategies, including providing comprehensive information about side effects, offering alternative formulations (e.g., intravenous iron for severe cases or intolerance), and ensuring consistent monitoring of treatment effectiveness and patient experience (Piskin et al., 2022).

Prenatal care challenges in the DRC contribute significantly to preventable maternal and child deaths and long-term health consequences (SEMI-IMA-UKaid, 2024). A strong and accessible prenatal care system is fundamental to reducing maternal and infant mortality, promoting healthy pregnancy and childbirth, and improving community well-being (Souza et al., 2024). Sustained investment in infrastructure, human resources, safety, quality training, and community involvement is essential for strengthening antenatal care and improving public health outcomes in the DRC (Duff et al., 2024).

DISCUSSION

Reiteration of key findings

The study focuses on the incidence of iron-deficiency anemia in pregnant women, particularly those previously diagnosed with microcytic hypochromic anemia. It highlights significant associations found, especially between iron-deficiency anemia and maternal characteristics including gravidity, parity, socioeconomic status, dietary habits, and adherence to iron supplementation. The high incidence of microcytic hypochromic anemia among anemic pregnant women, at 2.54%, is a crucial point, reinforcing the link between this morphological type of anemia and iron deficiency.

The 2.54% incidence of iron-deficiency anemia in pregnant women may seem low but underscores the importance of regular monitoring and highlights a significant public health concern and the need for interventions (Obianeli et al., 2024). This figure shows that, even in apparently well-resourced populations, a notable minority of pregnant women remain at risk of iron-deficiency anemia, with serious consequences for both mother and baby (Benson et al., 2021).

Interpretation and comparison with existing literature

Cox stratified multivariate regression analysis highlights several key factors influencing iron-deficiency anemia in pregnant women (Sugesti & Sansuwito, 2025). This powerful statistical technique identifies factors affecting time to event while accounting for potential confounders and varying baseline risks across strata (Islamiyati & Tinungki, 2023). Thus, interventions can be targeted at those most likely to benefit (Wang et al., 2025), making strategies more effective and efficient by concentrating resources. This high-risk approach differs from population-wide strategies aiming to modify risk factors across the entire population (Pieters et al., 2023).

When red blood cells are microcytic (smaller than normal) and hypochromic (paler than normal), it strongly suggests insufficient hemoglobin and, often, inadequate iron to produce it. In pregnant women, with increased iron requirements, this morphology directly indicates potential iron deficiency (Li et al., 2022b). Hypochromic microcytic anemia is not just a general sign of anemia; its presence specifically reinforces suspicion of iron deficiency in pregnancy, highlighting increased iron demand and the need for supplementation. This confirms its clinical value for rapid diagnosis and management in this vulnerable population (Obeagu, 2024b).

Because microcytic hypochromic anemia is relatively easy to identify through routine, inexpensive complete blood counts (CBC), it serves as an excellent initial screening tool. Detection of this anemia signals the need for further investigation (Eliwa et al., 2025). It acts as a preliminary marker of iron deficiency, prompting additional testing with serum ferritin—the gold standard for assessing iron stores—low levels of which confirm iron-deficiency anemia. This approach avoids unnecessary ferritin testing in individuals without red cell abnormalities while ensuring proper assessment of high-risk groups (Rusch et al., 2023).

Iron-deficiency anemia during pregnancy is linked to several adverse maternal and fetal outcomes (Obianeli et al., 2024), including an increased risk of pre-eclampsia later in pregnancy (Yang et al., 2024). Pregnant women are a key target group for malaria control interventions (USAID, 2015), as protective immunity developed in areas of high or

moderate transmission weakens during pregnancy (Alvarez et al., 2005).

Since microcytic anemia is a common hematological complication in pregnancy, with iron deficiency being the predominant cause, effective diagnosis relies on combined laboratory tests to distinguish iron deficiency from other causes, allowing precise, personalised therapeutic approaches (Petraglia et al., 2024).

Lower body mass index (BMI) in early pregnancy is associated with a higher risk of iron-deficiency anemia (Mohamed Ahmed Ayed et al., 2021). Pregnant women with lower BMI are more likely to develop iron-deficiency anemia (Kadhim, 2023).

In the absence of scientific evidence, it is generally assumed that married women's risk of iron-deficiency anemia during pregnancy may have more severe health implications than any single factor alone (Das et al., 2024; Liyew et al., 2021).

Lack of iron supplementation in pregnant women is a risk factor for maternal iron-deficiency anemia (Babah et al., 2024). However, the benefits of supplementation outweigh the risks associated with iron deficiency (Georgieff & Krebs, 2020). Pregnant women are particularly vulnerable to malaria infection and iron deficiency, increasing risks of maternal anemia, maternal death, and spontaneous abortion (Unger et al., 2022). Adequate iron intake during pregnancy results in better outcomes (Quezada-Pinedo et al., 2021). Women living in regions with prevalent iron deficiency should benefit from systematic iron supplementation during pregnancy, even where malaria is endemic (Tang & Krebs, 2019). In these settings, iron administration should be combined with malaria control measures such as insecticide-treated bed nets and accessible malaria diagnosis and treatment services (Elmardi et al., 2021; White, 2018; Roll Back Malaria, 2008).

Soil-borne helminth infections are widespread, contributing significantly to malnutrition and morbidity in poor regions. Over a billion people worldwide are affected, resulting in an annual loss of 39 disability-adjusted life years (Chen et al., 2024). Hookworm infection is a major cause of iron-deficiency anemia, especially in heavily infected pregnant women living in underdeveloped tropical countries (Tiremo & Shibeshi, 2023). Hookworm infestation is among the most common helminth infections and contributes substantially to anemia burden globally. In endemic regions, up to 90% of pregnant women are anemic (Caldrer et al., 2022).

Clinical Implications

Systematic screening for anemia during pregnancy, especially in women with microcytic hypochromic anemia, is crucial due to the potential negative impacts of iron deficiency on both maternal and fetal health. Appropriate diagnostic tools, such as complete blood counts with erythrocyte indices and serum ferritin levels, are essential for accurate diagnosis and effective management (Obeagu et al., 2025b). Although oral iron is the standard first-line treatment, patient compliance issues due to gastrointestinal side effects are common, and intravenous iron may be necessary for those who do not respond to or tolerate oral iron. Iron supplementation strategies need to be tailored to the needs and circumstances of each patient, considering both the benefits and potential difficulties of oral and intravenous therapies (DeLoughery et al., 2024). Identifying iron-deficient pregnant women is crucial for targeted interventions and nutritional counselling to address iron deficiency anemia. This proactive approach enables the development and implementation of tailored strategies that can improve maternal and infant health outcomes (Lewkowitz & Tuuli, 2022).

Study Limits

As a cohort study, although strong associations may be shown, proving direct causality may be difficult (Karamitros et al., 2025). The study acknowledges limitations in controlling all confounding factors, specifically other micronutrient deficiencies, genetic predispositions to anemia, and pre-existing medical conditions. These factors could potentially influence the study's results, making it difficult to isolate the specific effects under investigation (Brickley, 2024). In a prospective matched cohort study examining the incidence of iron-deficiency anemia following microcytic hypochromic anemia during pregnancy, controlling for confounders is vital since anemia has various causes, not all leading to iron-deficiency anemia. Pregnancy also introduces unique physiological changes and risk factors influencing anemia development and progression (Baradwan et al., 2018).

Patient-reported adherence to iron supplementation is often unreliable, representing a significant limitation in studies and interventions addressing iron deficiency. This unreliability stems from factors such as forgetfulness, supplement side effects, and negative perceptions (Saeed et al., 2024).

Future Research

- Randomized controlled trials (RCTs) are crucial for evaluating the efficacy and safety of different iron supplementation regimens. These trials compare the effects of various iron treatments (e.g., different iron salts, doses, or administration schedules) against a control group (e.g., placebo or alternative treatment) to determine the most effective and well-tolerated approach (Banerjee et al., 2024).
- Long-term health follow-up studies of mothers and children affected by HIV during pregnancy are essential for understanding the virus's impact and the effectiveness of interventions. These studies track health outcomes over time, including HIV transmission, growth and development, and the long-term effects of antiretroviral therapy (ART). Research in this area helps refine prevention and treatment strategies, aiming to eliminate mother-to-child HIV transmission (Yang et al., 2019).
- Research indicates that screening for iron-deficiency anemia (IDA) can be cost-effective, particularly in vulnerable populations, though optimal strategies vary by healthcare setting and target group. For instance, screening at a ferritin threshold of 25 µg/L has been identified as cost-effective in some studies. However, decisions to implement screening programs should be based on local IDA prevalence, available resources, and potential health impacts (Allen et al., 2025; Yang et al., 2019).
- Further research is needed into the precise molecular mechanisms by which IDA affects maternal and fetal health.

Study Limitations

Pregnant women may have other health issues influencing their iron status, which may not be fully controlled or considered. Although matching controls for known confounders such as diet quality and genetic predisposition, these factors may still influence iron-deficiency anemia development and may not be fully accounted for by statistical matching (Bösch et al., 2024). Pregnancy considerably alters iron requirements and metabolism. Even women without a history of microcytic hypochromic anemia may develop iron-deficiency anemia due to increased fetal needs and expanded plasma volume. This physiological change complicates isolating the specific impact of prior microcytic hypochromic anemia (Camaschella, 2019). In the absence of randomized clinical trials—which provide better estimates, are less subject to selection bias, and allow fuller interpretation (Soni et al., 2019)—this study helps fill gaps in maternal anemia research, which has traditionally focused on anemia severity rather than iron deficiency as its cause (Raut & Hiwale, 2022).

CONCLUSION

The study revealed a significantly high incidence of iron-deficiency anemia in pregnant women previously diagnosed with hypochromic microcytic anemia. This suggests that this type of anemia is a major risk factor for subsequent iron deficiency anemia, even if the initial etiology was not exclusively iron deficiency. The results highlight the importance of careful monitoring and regular screening for iron deficiency in pregnant women, especially those with a history of hypochromic microcytic anemia. Early detection allows for rapid and preventive intervention.

Using a matched design controlled many potential confounders, strengthening internal validity and enabling more accurate isolation of the relationship between hypochromic microcytic anemia and later iron deficiency anemia. Based on this study, it is strongly recommended that specific management protocols be incorporated for pregnant women with hypochromic microcytic anemia. These could include routine measurement of iron status markers (serum iron, ferritin, total transferrin-binding capacity) after initial diagnosis, even if iron supplementation is ongoing; prophylactic iron supplementation potentially at higher doses or for longer durations depending on lab results; and patient education on the importance of dietary iron intake and signs of iron deficiency.

Further research could explore the effectiveness of different preventive strategies in this at-risk population and the long-term impact of recurrent iron deficiency anemia on maternal and fetal outcomes. Genetic studies on predispositions to iron deficiency anemia in these patients would also be valuable. This matched prospective cohort study highlights the vulnerability of pregnant women with prior hypochromic microcytic anemia to developing iron deficiency anemia later. These findings have important clinical implications, underscoring the need for increased vigilance and targeted interventions to prevent and manage this common and potentially serious pregnancy complication. The results confirm the hypothesis that iron-deficiency anemia incidence is higher in pregnant women with microcytic hypochromic anemia than in those without it.

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Availability of Data and Materials: Data sets used and/or analysed during the study are available from the corresponding author upon reasonable request.

Ethical Approval: **Ethics Approval and Consent to Participate** The primary data originate from a survey approved by the Superior Institute of Technical Medicine of Kinshasa Bioethics Committee (approval number 0038/CBE/ISTM/KIN/RDC/PMBBL/29.11.2023). All methods were performed in accordance with relevant guidelines and regulations. Informed consent was obtained from all subjects and/or their legal guardians.

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