

# Contributions of malaria, helminths, HIV and iron deficiency to anaemia in pregnant women attending ante-natal clinic in SouthWest Nigeria

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## Abstract

**Background:** Iron deficiency is a dominant source of anaemia in many settings. To evaluate the key cause of anaemia in the study area, the prevalence of anaemia due to major public health diseases was compared with anaemia due to iron deficiency.

**Methods:** Pregnant women were recruited from ante-natal (n=490) and HIV clinics (n=217) with their personal data documented using a questionnaire. Microscopy of Giemsa-stained thick smears was used for detection of malaria parasites while helminths in stools were detected using direct smear method. Haematocrit values were determined by capillary method. Serum ferritin levels were determined using enzyme-linked immunosorbent assay. Data was analysed using SPSS version 22.0.

**Results:** The mean age of the recruited women was 28.6±5.4 years old. There were 68.1% cases of anaemia of which 35.5% was due to infections only predominantly HIV and malaria, 14.9% from unknown sources while anaemia due to iron deficiency only was 7.1%.

**Conclusion:** It can safely be inferred that malaria and HIV predispose to anaemia than iron deficiency in the study area. Although pregnant women are dewormed and given IPTp for helminths and malaria treatment respectively, there should be complementary routine malaria screening at ANC visits for those with HCT values <33% and those infected with HIV.

**Keywords:** Iron deficiency anaemia; antenatal care; *Plasmodium*; helminth; pregnant women; HIV.

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## Introduction

Anaemia is a state of reduced number of red blood cells resulting in lower oxygen-carrying capacity of the cells which invariably affects the body's physiologic functions. According to World Health Organization (WHO), haemoglobin level less than 11g/dl in pregnancy is an indication of anaemia<sup>1</sup>. Anaemia can be caused by nutritional deficiencies, inflammation, parasitic infections, blood loss and haemoglobinopathies

with iron deficiency being reported as the major cause of anaemia<sup>2,3</sup>. Globally, an estimated 41.8% pregnant women are reported to be anaemic and in about half of these women, anaemia is presumed to be due to iron deficiency<sup>4</sup>. High prevalence of anaemia in pregnancy led to the recommendation of the WHO guideline for daily iron and folic acid supplementation in pregnancy to reduce the risk of low birth weight babies, maternal anaemia and iron deficiency<sup>4</sup>. However, overview of studies conducted in Nigeria have reported persistent high prevalence of anaemia among pregnant women<sup>5-8</sup>. This necessitated the need to evaluate other underlying causes of anaemia.

Malaria, helminths and human immunodeficiency virus (HIV) are common diseases in poor countries and have been associated with anaemia in pregnancy<sup>5,9-17</sup>. The burden of anaemia associated with these infections should be minimal if there were strict adherences to the WHO guidelines for use of Intermittent Preventive Treatment in pregnancy (IPTp) for control of malaria<sup>18</sup> and deworming regimen to reduce burden of helminthiasis in pregnancy<sup>19</sup>. However, poor compliance with IPTp usage has been reported<sup>15,20,21</sup> thus proposing a multidimensional approach to resolving the burden of anaemia in pregnancy. The focus of this study was therefore to conduct a comparative analysis of the relative contribution of these infections and iron deficiency to anaemia among pregnant women in SouthWest Nigeria.

## Methods

### Study site, design and study population

This study was carried out in Adeoyo Maternity Hospital, a secondary healthcare facility and Agbongbon Primary Healthcare Centre in Ibadan, Southwest Nigeria. They are government owned and serves the local inhabitants who are predominantly petty traders and home makers. The average attendances at each ante-natal clinic visit of these hospitals are about 120 and 25 pregnant women respectively.

This is a cross-sectional study with inclusion and exclusion criteria as follows:

**Inclusion criteria:** Pregnant women between 18-45 years old were recruited at their first antenatal visit. Confirmed HIV infected pregnant women were recruited from the HIV clinic situated within the secondary healthcare facility. Informed consent was obtained from all the women at recruitment. Only patients from whom both blood and stool samples were collected were included in the study.

**Exclusion criteria:** Pregnant women with observable

complications were excluded. At the regular antenatal clinic, pregnant women that tested positive for HIV during the hospital routine tests at booking were excluded. They were referred to HIV clinic for confirmatory testing.

Overall, 707 pregnant women were recruited. They comprised of 490 HIV negative pregnant women recruited from the ante-natal clinics (ANCs) and 217 HIV positive from the HIV clinic. Well-structured close-ended questionnaires were administered by trained data collectors in the native language of the subjects (Yoruba) as majority of them were semi-literate. These provided information on socio-demographic details, educational history, sanitary practices and living conditions.

Ethical approvals for this work were obtained from Oyo State Ministry of Health and the University of Ibadan/University College Hospital Ethical Review Committees. Individual informed consent was obtained from each enrollee before study related procedures were carried out.

### Sample size

The sample size was calculated using the formula below at a 25% expected prevalence rate (P) and a precision (d) of 0.05 (22).

$$n = \frac{Z^2 P (1-P)}{d^2}$$
$$n = \frac{(1.96)^2 \times 0.25 (1-0.25)}{(0.05)^2} = 286$$

A minimum of 314 pregnant women were proposed to be recruited having added 10% of the calculated value to suffice for unexpected outcomes.

### Anaemia assessment

#### Haematocrit (HCT)

Venous blood was withdrawn into heparinized capillary tube and sealed at one end with plasticine. The tubes were spun at 3000 rpm for 10 mins in a microhaematocrit centrifuge (Hawksley Ltd, Lancing, UK) and read with Hawksley haematocrit reader. In pregnancy HCT levels <33% was taken as an indication of anaemia<sup>1</sup>.

#### Serum ferritin (SF)

Five millilitres of blood was collected by venipuncture into plain tubes and spun at 3000g for 5 mins in a centrifuge. Serum samples were stored at -20°C until when the ferritin levels were determined using enzyme linked immunosorbent assay (ELISA) kits obtained from Assaypro, USA and the assay was performed according to manufacturer's instructions. Standard curves were generated and data interpreted using Curve Expert 1.4 software.

## Parasitological assessment

Thick blood smears were prepared on microscope slides for each participant and allowed to air dry away from direct sunlight. Dried smears were stained with fresh Giemsa stain at pH 7.2 using standard procedures and were subsequently screened under light microscope at x1000 magnification for presence and quantification of asexual stage of malaria parasite. Definitive parasite density was calculated as number of parasites counted against about 200 white blood cells using an assumed white blood cell count of 8000/mm<sup>3</sup> <sup>23</sup>. Intestinal helminths were identified by microscopic examination of saline preparation of stool on a glass slide covered with cover slip. Kato-Katz technique was used for quantification of helminth ova <sup>24</sup>.

## Data analysis

Statistical package, SPSS version 22.0 (IBM® SPSS®, Chicago, IL, USA) was used for the data analysis. Prevalence values were determined using descriptive statistics.

Pearson Chi-Square was used to test for associations while Student t-test was used to determine significant differences in mean values between two groups. Significant level was set at p<0.05. Iron deficiency anaemia was defined as HCT <33% and serum ferritin values <12ng/ml or <30ng/ml based on infection status <sup>25</sup>. Using WHO reference values, SF values <12ng/ml was used as cut-off among those without infection and the cut-off was raised to 30ng/ml for those infected <sup>26</sup>.

## Results

The mean age of the 707 recruited pregnant women was 28.6±5.4 yrs old. They were mostly multigravids (75.1%) and secondary school leavers (56.9%). The major occupation of the recruited women was trading mostly in food and food items (54.4%). Drawing water from deep wells with the use of bucket and rope was the major source of portable water and 5.4% of the women had no toilet in the house. Table 1 shows details of the socio-demographic status of the women.

**Table 1: Socio-demographic characteristics of recruited pregnant women**

Characteristics	N	Status	n (%)
Age	700	< 20 years	22 (3.1)
		20-34 years	564 (80.6)
		≥ 35 years	114 (16.3)
Gravidity	695	Primigravidae	173 (24.9)
		Multigravidae	522 (75.1)
Level of education	703	None	5 (0.7)
		Primary/quranic	138 (19.6)
		Secondary	400 (56.9)
		Post-secondary	160 (22.8)
Occupation	702	Student/Unemployed	52 (7.4)
		Petty trader	382 (54.4)
		Primary school teacher/Junior civil servant/Artisan	188 (26.8)
		High school teacher/Middle level civil servant/Middle businessman	62 (8.8)
		Major business person/Professional	18 (2.6)
		Source of portable water	706
		Well with pump	40 (5.7)
		Well with bucket and rope	452 (64.0)
		Pipe-borne water	72 (10.2)
		Borehole	141 (20.0)
Toilet facility	705	None	38 (5.4)
		Pit latrine	315 (44.7)
		Water system	352 (49.9)

Sixty-seven (9.4%) had co-infections (*Plasmodium* and helminths – 1.1%, *Plasmodium* and HIV – 7.4%, HIV and helminths – 0.8%, *Plasmodium*, HIV and helminths – 0.1%) while 258 (36.4%) had single infections (*Plasmodium* – 7.5%, Helminth - 6.6%, HIV – 22.3%). A higher proportion (54%) of the recruited women were uninfected. The helminths found were *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms. The mean haematocrit value (HCT) of the recruited women was

31.7±5.4% and 387 (57.6%) of the pregnant women were anaemic (mild anaemia – 24.0%, moderate anaemia – 32.0%, severe anaemia – 1.6%). Relative to the mean HCT of the uninfected group (32.9 ± 5.6%), HCT was lower in all the infected groups and these were significantly lower among those with co-infection of *Plasmodium* and HIV (29.7 ± 4.8%) and those with single infections of HIV (29.3 ± 5.0%) and *Plasmodium* (31.0 ± 4.0%) as shown in Table 2.

**Table 2: Mean HCT values and anaemia classification in relation to infection status.**

Groups	n	Mean ± SD	%	Mild	Moderate	Severe
anaemia						
PF	53	31.0 ± 4.0 <sup>a</sup>	60.4	16 (30.2%)	16 (30.2%)	--
H	44	32.2 ± 5.0	54.5	11 (25.0%)	13 (29.5%)	--
HIV	143	29.3 ± 5.0 <sup>b</sup>	76.2	39 (27.3%)	64 (44.8%)	6 (4.2%)
PF+H	8	29.9 ± 4.7	75	3 (37.5%)	3 (37.5%)	--
H+HIV	5	30.6 ± 4.0	80	2 (40.0%)	2 (40.0%)	--
PF+HIV	48	29.7 ± 4.8 <sup>c</sup>	72.9	15 (31.3%)	18 (37.5%)	2 (4.2%)
PF+H+HIV	1	27.0	100	--	1 (100%)	--
Uninfected	370	32.9 ± 5.6 <sup>a,b,c</sup>	47.6	75 (20.3%)	98 (26.5%)	3 (0.8%)

$\chi^2 = 54.955, P < 0.0001$

**Key:** PF – *P. falciparum*, H – Helminth, SD – standard deviation

Similar superscripts show significant difference at  $p < 0.05$

A significantly higher proportion of those with *P. falciparum* infection (67.3%) and HIV (75.6%) were

anaemic while the proportion of those with helminth infection and who had anaemia (60.3%) was not statistically significant from those without anaemia (Table 3).

**Table 3: Association between anaemia and infections**

Anaemia	PF positive	PF negative	Total	$\chi^2$	P-value
Present	74 (67.3%)	313 (55.7%)	387 (57.6%)	5.050	0.025*
Absent	36 (32.7%)	249 (44.3%)	285 (42.4%)		
Total	110 (100%)	562 (100%)	672 (100%)		
	H positive	H negative	Total		
Present	35 (60.3%)	352 (57.3%)	387 (57.6%)	0.197	0.657
Absent	23 (39.7%)	262 (42.7%)	285 (42.4%)		
Total	58 (100%)	614 (100%)	672 (100%)		
	HIV positive	HIV negative	Total		
Present	149 (75.6%)	238 (50.1%)	387 (57.6%)	37.158	<0.0001*
Absent	48 (24.4%)	237 (49.9%)	285 (42.4%)		
Total	197 (100%)	475 (100%)	672 (100%)		

**Key:** PF – *P. falciparum*, H – Helminth \*significant at p<0.05

From the larger population of 707 recruited pregnant women, serum samples of 141 women were selected across the infection groups for determination of serum ferritin levels and the results were grouped as iron deficient only, or iron deficiency anaemia. As shown in Table 4, there was 68.1% (96/141) prevalence of anaemia

and 26.0% (25/96) of the anaemia cases were due to iron deficiency. A small proportion of those who were infected with *Plasmodium*, helminths or HIV were also iron deficient. Hence, the test of associations between these infections and iron deficiency were not statistically significant. Similarly, the test of association between anaemia and iron deficiency was not significant.

**Table 4: Association between iron deficiency, anaemia and infections.**

	PF positive	PF negative	Total	$\chi^2$	P-value
Iron deficient	10 (19.2%)	21 (23.6%)	31 (21.4%)		
Normal	42 (80.8%)	68 (76.4%)	110 (78.6%)		
Total	52 (100%)	89 (100%)	141 (100%)	0.365	0.546
	H positive	H negative	Total		
Iron deficient	4 (17.4%)	27 (22.9%)	31 (22%)		
Normal	19 (82.6%)	91 (77.1%)	110 (78%)		
Total	23 (100%)	118 (100%)	141 (100%)	0.338	0.561
	HIV positive	HIV negative	Total		
Iron deficient	13 (24.5%)	18 (20.5%)	31 (22%)		
Normal	40 (75.5%)	70 (79.5%)	110 (78%)		
Total	53 (100%)	88 (100%)	141 (100%)	0.320	0.572
	Anaemia present	Anaemia absent	Total		
Iron deficient	25 (26.0%)	6 (13.3%)	31 (22.0%)		
Normal	71 (74.0%)	39 (86.7%)	110 (78.0%)		
Total	96 (100%)	45 (100%)	141 (100%)	2.885	0.089

**Key:** PF – *P. falciparum*, H – Helminth

Table 5 shows the distribution of iron deficiency and anaemia according to the infection status. The prevalence of iron deficiency anaemia among the pregnant women irrespective of the infection status was 17.7% (25/141) while the prevalence of anaemia without iron deficiency was 50.4% (71/141). The prevalence of iron deficiency anaemia without the compounding ef-

fect of infection was 7.1% (10/141). The prevalence of anaemia associated with infection only was 35.5% (50/141). The prevalence of anaemia due to unknown sources (absence of infection and no iron deficiency) was 14.9% (21/141). Iron deficiency anaemia was more prevalent among those infected with HIV only and the uninfected group.

**Table 5: Prevalence of anaemia and iron deficiency in relation to infection status**

	n	Normal HCT/ ferritin levels	Anaemia only	Iron deficiency only	Iron deficiency anaemia
<i>Plasmodium</i> only	23	8 (34.8%)	12 (52.2%)	2 (8.7%)	1 (4.3%)
Helminth only	18	9 (50%)	6 (33.3%)	2 (11.1%)	1 (5.6%)
HIV only	29	4 (13.8%)	18 (62.1%)	-	7 (24.1%)
<i>Plasmodium</i> /Helminth	5	1 (20%)	3 (60%)	-	1 (20%)
<i>Plasmodium</i> /HIV	24	7 (29.2%)	11 (45.8%)	1 (4.2%)	5 (20.8%)
Uninfected	42	10 (23.8%)	21 (50%)	1 (2.4%)	10 (23.8%)
Total	141	39 (27.7%)	71 (50.4%)	6 (4.3%)	25 (17.7%)

## Discussion

Anaemia is a common complication of pregnancy in tropical African countries where poverty related infectious diseases are endemic. We therefore evaluated the prevalence of anaemia due to infection and iron deficiency to establish the need to complement the current interventions of iron supplementation and intermittent preventive treatment of malaria in pregnancy.

The prevalence of anaemia among the pregnant women was 68.1%. Similar findings of more than half of the pregnant women being anaemic have been reported within and outside Nigeria<sup>5-7,9,11,27</sup>. However, some other studies reported <30% prevalence of anaemia among pregnant women<sup>8,28-30</sup>. Data showed that *Plasmodium*, helminth and HIV infections caused reduction in HCT values. The differences were found to be significant in *Plasmodium* and HIV infections relative to the uninfected group. Some studies have likewise reported reduction in HCT values among those with *Plasmodium* infection<sup>5,31</sup>. It is established that destruction of red blood cells, a key feature of malaria pathogenesis will predispose to anaemia<sup>32</sup>. Likewise, reduction in HCT values in HIV infections has been reported by some authors<sup>14,29</sup>. Moreover, there was a strong association between anaemia and *Plasmodium* and HIV infections. This supports earlier reports of a strong association between severity of parasitaemia and degree of anaemia<sup>5,11,14,33</sup>. Anaemia in HIV infection has been linked to progression of the disease, poor quality of life and

higher mortality<sup>34</sup>. Furthermore, there was no significant association in either of the three infections studied and iron deficiency. Although there is a deficit of data on SF levels among helminth infected pregnant women, a study among school children showed no significant association between iron deficiency and ascariasis as obtained in this study<sup>35</sup>. Another study among school children also found no significant association between iron deficiency and ascariasis. According to their findings, severe trichuriasis was the major contributor to iron deficiency<sup>36</sup>. Hookworms and *Trichuris* seem to be the major causes of iron deficiency<sup>36,37</sup>. Among the three predominant worms; hookworms, *Ascaris* and *Trichuris*, hookworms are more likely to be strongly associated to low serum ferritin levels because of their migratory behaviour leaving many bleeding spots leading to intestinal blood losses<sup>38</sup>. Likewise, there was no significant association between iron deficiency and anaemia in this present study. However, a study of pregnant women in Uganda showed a significant association between them<sup>33</sup>. The difference in findings may be due to variation in cut-off values for ferritin in these studies. A cut-off of < 12µg/l was used in this study for those without infection and <30µg/l for those with infection while they used <30µg/l as a general cut-off value. This study further substantiated the evidence of high prevalence of anaemia among pregnant women and infection has been identified as the major cause of anaemia in this setting. With the removal of confounders,

there was a low prevalence (7.1%) of iron deficiency anaemia (IDA) while the prevalence of infection associated anaemia was 35.5% with HIV infection being the highest contributor to anaemia. Contrary to this finding, iron deficiency is reportedly the dominant cause of anaemia in some settings<sup>27,36</sup>. This low prevalence of IDA showed that nutritional deficiency may not be a major public health issue among the pregnant women in this present study. Hence, strategic policies to reduce the burden of these infections in pregnancy is highly required as quite a number of malaria cases are undetected when there are no clinical symptoms. This was observed among these pregnant women at booking. Presently, there is no guideline for routine screening for malaria and helminths in ante natal clinics in healthcare facilities in Nigeria. Hence, there is likelihood of these women remaining untreated as the adopted intermittent presumptive treatment in pregnancy (IPTp) has recorded cases of poor compliance<sup>15,20,21</sup>. Furthermore, the effect of iron supplementation on reducing the burden of anaemia in pregnancy may be masked without concurrent treatment of underlying infection.

### Conclusion

The high burden of anaemia in the study population is largely due to the presence of infection and not deficiency of iron. Further research on the impact of treatment on prevalence of anaemia among pregnant women infected with malaria parasitaemia or helminthiasis may lay further credence to the need for routine screening during ante-natal visits.

### Conflict of interest

The authors declare no conflict of interest.

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