Malaria and hookworm co-infection and their effects on anaemia of pregnant women attending ante-natal clinic in University Teaching Hospital, Calabar, Nigeria

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ABSTRACT

Background: Malaria and hookworm infections independently have been known to cause anaemia. Coinfection of these two parasites can aggravated the morbidity of these infections with a resulting impact on anaemia especially in pregnancy. **Aims and Objective**: This work was aimed at assessing the prevalence of malaria and hookworm co-infection and their effects on anaemia among pregnant women in Calabar Metroplis. **Materials and Methods:** Giemsa stained film of venous blood was examined microscopically for malaria parasites while cyanmethaemoglobin method was used for haemoglobin level estimation. Stool samples were examined microscopically while Stoll's technique was used in counting the ova of hookworm. **Results:** 19(6.3%) of the subjects were infected with hookworm, 45(15.0%) with malaria, while 9(3.0%) were infected with both parasites. Severe anaemia of 4(44.4%) was observed amongst the test (infected) subjects while the control (uninfected) subjects had a severe anaemia of 3(1.0%). The highest prevalence of co-infections of malaria and hookworm 4(38.6%) occurred among the age group of 26-30years (p = 0.371). Subjects in their 3rd trimester had the highest prevalence of co-infection of 4(4.4%) (p = 0.412). The highest prevalence of severe anaemia 1(100.0%) occurred among the age group of 16-20years (p = 0.002). **Conclusion:** This work has shown a low prevalence of malaria and hookworm co-infection. The level of severe anaemia among infected pregnant women was more than that observed among subjects without any of the two infections.

Key words: Malaria, Hookworm, Co-infection, Anaemia, pregnant women, Calabar

INTRODUCTION

Malaria in pregnancy is a major contributor to adverse maternal and perinatal outcomes. Maternal malaria has been reported to be associated with up to 200,000 infant deaths yearly.[1] Malaria is a common cause of anaemia in pregnancy in both immune and non-immune individuals and is aggravated by poor socio-economic factors. Co-infection with hookworm and malaria is common in Africa,[2] but the exact number is unknown. Malaria and hookworm co-infection have been shown to have additive impact on anaemia resulting in adverse pregnancy outcomes.[3] There have been few studies on the impact of malaria and hookworm co-infection among pregnant women in rural communities where these parasites are endemic. Hookworms infect more than 600 million people worldwide and it's the leading cause of maternal and child morbidity in the developing countries of the tropics and subtropics.[4] It is estimated that a-third of all pregnant women in developing countries are infected with hookworm, 56% of all pregnant

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women in developing countries suffer from anaemia, while 20% of all maternal deaths are either directly or indirectly related to anaemia. *N. americanus* is the most common hookworm species worldwide, while *A. duodenale* is more geographically restricted. There is no known animal reservoir for *N. americanus* or *A. duodenale*.[5] In pregnant women, anemia caused by hookworm disease results in several adverse outcomes for both the mother and her infant, including low birth weight, impaired milk production, and increased risk of death for both the mother and the child. In children, chronic hookworm infection has been shown to impair physical and intellectual development, reduce school performance and attendance, and adversely affect future productivity and wage-earning potential.[6] This study was aimed at ascertaining the impact of malaria and hookworm co-infection and its relationship with anaemia among pregnant women attending Antenatal Clinic at University of Calabar Teaching Hospital (UCTH), Calabar.

MATERIALS AND METHODS

Study area
The study was carried out at University of Calabar Teaching Hospital (UCTH), Calabar, Cross River State, Nigeria. The University of Calabar Teaching Hospital is located on the latitude and longitude of $4^030'$ and $4^040'$N and $08^05'$and $8^015'$E respectively.[7] Calabar Municipality and Calabar-South Local Government Areas population density is estimated at 470, 950[8].

Study design
This was a cross sectional study of 300 pregnant women aged 12 years and above attending Antenatal Clinic in University of Calabar Teaching Hospital (UCTH), Calabar, Cross River State, Nigeria.

Inclusion criteria
Any pregnant woman who was aged 12 years and above at the time of study.

Any pregnant women who gave either written informed consent.

Exclusion criteria
Women aged 12 years and above who were not pregnant.

Any pregnant woman who had taken anti-malaria drug within two weeks prior to the study.

Any pregnant woman who had taken anthelminthics drug within two weeks prior to the study.

Any pregnant woman who was known HbSS-pregnant women.

Any pregnant woman known to have had chronic cardiac/kidney problems.

Questionnaire administration
Questionnaire was administered to the subjects.

Ethical clearance and informed consent
Ethical clearance was sought and obtained from University of Calabar Teaching Hospital health research and ethical committee. Informed or written consent was also obtained from the subjects prior to sample collection.

Control subjects
The pregnant women without both infections aged 12 years and above were used as control.

Samples collection
Five milliliter of blood sample was collected from each of the subjects by standard venipuncture method into EDTA container and mixed. That was used for the estimation of haemoglobin. One millilitre of blood from syringe non-EDTA, collected was used for *Plasmodium* detection. An aliquot of freshly passed stool sample was collected from each of the subjects into clean universal container. Both samples of blood and stool were also collected from the control subjects.

Processing of samples
Estimation of haemoglobin
Twenty microlitre of blood was collected from EDTA blood sample of each of the subjects and processed by cyanmethaemoglobin method for estimation of haemoglobin concentration.

Detection of malaria parasites microscopically
Detection and identification of malaria parasite from peripheral blood smear was done by Giemsa staining technique by World Health Organization (WHO) criteria.[9] Calculation of plasmodium parasitaemia density: number of parasites/WBC counted X 8000WBC/ul.[10] Also degree of anaemia was categorized using WHO[11] standard; mild anaemia Hb 10-11.9g/dl; moderate anaemia Hb 7-9.9g/dl; severe anaemia Hb 6.0-6.9g/dl.

Processing of stool sample
Diagnosis for intestinal parasites was done using direct wet mount microscopic examinations and formol ether concentration technique as described by Cheesbrough.[5]

Determination of hookworm density
Stoll's technique was used in counting ova of hookworm as described by Stoll.[12]

Statistical analysis
Data were analysed using SPSS version 20 statistical package (SPSS, Inc. Chicago, IL USA). A Chi-squared test was used

to examine the association between variables. The density of infections was determined by the use of the mean and standard deviation. P- value at 5% confidence interval of 95% was used to calculate the level of significance.

RESULTS

Of the 300 subjects examined, 19(6.3%) were infected with hookworm, 45(15.0%) had malaria parasitaemia, 9(3.0%) were infected with both hookworm and malaria parasites. Table 1 shows the prevalence of malaria and hookworm co-infections, haemoglobin levels and degree of anaemia by age. The highest level of co-infection 4(28.6%) occurred among the age of 26-30 years while age group 31-35years, 36-40years and 41-45years had the lowest prevalence of 0(0.0%). The difference was not statistically significant. (X^2 =5.383, (df)=2, p=0.371). Subjects in the age group 16-20 years had the highest mean malaria parasite count of 12266.0±0.0/µl of blood while age group 26-30 years had lowest mean parasite density of 2149.7±2813.3/µl of blood. The difference in distribution of mean parasite density was not statistically significant (X^2= 0.838, (df)=2, p=0.477). Subjects in the age group 16-20 years had the highest hookworm ova density of 2500.0±0.0 e/g of stool while age group of 21-25 years had the lowest hookworm ova density of 975.0±531.5 e/g of stool. The difference in distribution of mean parasite density was not statistically significant (X^2=3.675, (df)=2, p=0.091). The highest mean Hb of 8.9±0.9g/dl was observed among the age group of 26-30 years while age group of 16-20years had the lowest mean Hb of 6.0±0.0g/dl. The difference in mean Hb levels was statistically significant (X^2=0.835, (df)=2, p=0.479). The highest prevalence of severe anaemia 1(100.0%) occurred among the age group of 16-20 years while the lowest prevalence of severe anaemia 3(75.0%) was observed among the age group of 21-25 years. The difference was statistically significant (X^2 =7.687,(df)=1, p=0.002). Among the control subjects, the highest mean Hb levels of 11.0±1.9g/dl occurred among the age group of 41-45 while the lowest mean Hb of 9.3±2.2g/dl occurred among the age of 16-20years. The difference in mean Hb was statistically significant (X^2=3.306, (df)=5, p=0.007). Those with age group of 16-20 years had the highest prevalence of severe anaemia 1(4.8%) while age group of 21-25years had lowest prevalence of severe anaemia 1(1.7%). The difference was not statistically significant (X^2 =7.425,(df)=2, p=0.191). Table 2 shows the prevalence of malaria and hookworm co-infections, haemoglobin levels and degree of anaemia by trimesters. Of 300 subjects, both infections of 4(4.4%) occurred among the pregnant women in their 3rd trimester while those in their 1st trimester recorded 0(0.0%). The difference in prevalence of co-infection according to trimesters was not statistically

Table 1: The prevalence of malaria and hookworm co-infection, haemoglobin (Hb) levels and degree of anaemia by age

		Test (infected subjects)							Control (uninfected subjects)				
Age group (yrs)	No examined	No (%) with both infections	Mean malaria count±SD/µl of blood	Mean hookworm ova count±SD e/g of stool	Mean Hb±SD g/dl	No (%) with mild anaemia	No (%) with moderate anaemia	No (%) with severe anaemia	No (%) without both infections	Mean Hb±SD g/dl	No (%) with mild anaemia	No (%) with moderate anaemia	No (%) with severe anaemia
16 – 20	22	1 (4.5)	12266.0+0.0	2500.0+0.0	6.0+0.0	-	-	1 (100.0)	21 (95.5)	9.3+2.2	6 (28.6)	7 (33.3)	1 (4.8)
21 – 25	64	4 (6.3)	11117.9+1475.9	975.0+531.5	7.9+2.9	-	-	3 (75.0)	60 (93.8)	10.6+1.4	25 (41.7)	16 (26.7)	1 (1.7)
26 – 30	114	4 (28.6)	2149.7+2813.3	1075.0+666.0	8.9+0.9	3 (75.0)	3 (75.0)	-	110 (96.5)	10.8+1.6	56 (50.9)	20 (18.1)	-
31 – 35	68	0 (0.0)	-	-	-	-	-	-	68 (100.0)	10.7+1.3	32 (47.1)	15 (22.1)	-
36 – 40	29	0 (0.0)	-	-	-	-	-	-	29 (100.0)	10.4+1.6	13 (44.8)	6 (20.7)	1 (3.4)
41 – 45	3	0 (0.0)	-	-	-	-	-	-	3 (100.0)	11.0+1.9	1 (33.3)	1 (33.3)	-
Total	300	9 (3.0)	7259.6+10402.3	1188.9+666.1	8.1+2.1	2 (22.2)	3 (33.3)	4 (44.4)	291 (97.0)	10.7+1.4	133 (45.7)	65 (22.3)	3 (1.0)
P-value		5.383	0.838	3.675	0.835	1.406	5.625	7.687	P-value	3.306	6.852	4.223	7.425
		0.371	0.477	0.812	0.479	0.495	0.060	0.002		0.007	0.232	0.518	0.191

Grading of Anaemia by WHO (2008): Mild anaemia PCV 30-36%/Hb 10-11.9g/dl; Moderate anaemia PCV 21-30%/Hb 7-9.9g/dl; Severe anaemia PCV 18-21%/Hb 6.0-6.9g/dl

Table 2: The prevalence of malaria and hookworm co-infection, haemoglobin (Hb) levels and degree of anaemia by trimesters

Age group (yr)	No examined	No (%) with both infections	Mean malaria count + SD/µl of blood	Mean hookworm ova count + SD e/g	Mean Hb + SD g/dl	No (%) with mild anaemia	No (%) with moderate anaemia	No (%) with severe anaemia	No (%) without both infections	Mean Hb + SD g/dl	No (%) with mild anaemia	No (%) with moderate anaemia	No (%) with severe anaemia
1st	39	0 (0.0)	-	-	-	-	-	-	39 (100.0)	11.0 + 1.5	17 (43.6)	7 (17.9)	1 (2.6)
2nd	171	4 (2.9)	12430.0 + 11869.7	1500.0 + 659.5	6.6 + 0.8	-	1 (20.0)	4 (80.0)	166 (97.1)	10.6 + 1.4	79 (47.6)	40 (24.1)	2 (1.2)
3rd	90	4 (4.4)	796.6 + 668.9	800.0 + 489.9	9.9 + 1.6	2 (22.1)	2 (50.0)	-	86 (95.6)	10.6 + 1.3	37 (43.0)	18 (20.9)	-
Total	300	9 (3.0)	7259.6 + 10402.3	1188.9 + 666.1	8.1 + 2.1	2 (22.2)	3 (33.3)	4 (44.4)	291 (97.0)	10.7 + 1.4	133 (45.7)	65 (22.3)	3 (1.0)
P-value		1.772	3.727	3.098	16.727	1.406	0.900	5.706	P-value	1.461	1.455	1.276	1.304
		0.412	0.950	0.122	0.005	0.236	0.405	0.016		0.234	0.234	0.190	0.524

Grading of Anaemia by WHO, (2008): Mild anaemia PCV 30-36%/Hb 10-11.9g/dl; BModerate anaemia PCV 21-30%/Hb 7-9.9g/dl; Severe anaemia PCV 18-21%/Hb 6.0-6.9g/dl

significant(X^2=1.772, df=2, p=0.412). The highest mean parasite density of 12430.0±1186.7/µl of blood occurred among subjects in their 2nd trimester while the lowest mean parasite density of 796.6±668.9/µl of blood was observed among the subjects in their 3rd trimester. The difference in distribution of mean malaria parasite density according to the trimesters of pregnancy was not statistically significant ((X^2=3.727, (df)=1, p=0.950). The highest hookworm ova density of 1500.0±659.5 e/g of stool occurred among pregnant women in their 2nd trimester while the lowest hookworm ova density of 800.0±489.9 e/g of stool occurred among the subjects in their 3rd trimester. The difference in distribution of mean parasite density according to the trimesters was not statistically significant (X^2=3.098, (df)=0, p=0.122). The highest mean Hb levels of 9.9±1.6g/dl occurred among the pregnant women in their 3rd trimester while the lowest mean Hb levels of 6.6±0.9g/dl was observed among the subjects in their 2nd trimester. The difference in the mean Hb was statistically significant ((X^2=16.727,(df)=0,p=0.005). Severe anaemia was only observed in 2nd trimester prevalence of 4(80.0%). Amongst control subjects, the highest mean Hb level of 11.0±1.5g/dl was observed among the subjects in their 1st trimester while the lowest mean Hb of 10.6±1.3g/dl was observed among subjects in their 3rd trimesters. The difference was not statistically significant (X^2=1.461,(df)=2, p=0.234).

Table 3 show the prevalence of malaria parasite and hookworm infections, haemoglobin levels and degree of anaemia by age. The aged group of 16-20years recorded the highest prevalence rate of 6(27.2%), while age group of 41-45years recorded the lowest prevalence of 0(0.0%). The difference in prevalence of malaria parasite was not statistically significant (X^2=3.069, (df)=4 and p=0.689).The highest mean parasite densities of the subjects was recorded amongst the age group of 31-35years with the highest parasites mean density of 5025.5±3252.6/µl of blood while the lowest mean parasite density of 674.9±910.3/µl of blood was observed among the age group of 26-30 years. The difference in distribution of mean parasite density was not statistically significant (X^2=0.868, (df)=4, p=0.491). Table 4 shows the prevalence of malaria parasite and hookworm infections, haemoglobin levels and degree of anaemia by trimesters. Of 300 subjects, 45(15.0%) had malaria parasitaemia. The highest prevalence of malaria parasite infection of 20(22.2%) was observed among the pregnant women in their 3rd trimester while the lowest prevalence of 3(7.7%) was observed among the subjects in their 1st trimester. The difference in prevalence of malaria infection according to trimester was not statistically significant (X^2=4.310, (df)=2 and p=0.116). The highest mean parasite density of 2592.1±8903.0/µl of blood was observed within the pregnant women in their 3rd trimester

Table 3: The prevalence of malaria parasites infection, haemoglobin (Hb) levels and degree of anaemia by age Test (Infected subjects)

Age group (yrs)	No examined	No (%) with malaria parasite infections	No (%) with hookworm infection	Mean hookworm ova count±SD e/g	Mean parasite count±SD/µl of blood	Mean Hb±SD g/dl	No (%) with mild anaemia	No (%) with moderate anaemia	No (%) with severe anaemia
16 – 20	22	6 (27.2)	1 (4.5)	2220.0+0.0	3823.7+7746.8	7.8+1.2	-	5 (83.3)	1 (16.7)
21 – 25	64	7 (10.9)	2 (3.1)	1500.0+1131.4	816.2+751.6	9.6+2.6	1 (14.3)	3 (42.9)	1 (14.3)
26 – 30	114	18 (15.8)	9 (7.9)	1533.3+886.0	674.9+910.3	10.6+1.4	10 (55.6)	5 (27.8)	-
31 – 35	68	9 (13.2)	5 (7.4)	1140.0+559.5	5025+3252.6	10.7+0.9	7 (77.8)	2 (22.2)	-
36 – 40	29	5 (17.2)	2 (6.9)	115.0+70.7	879.8+1242.1	11.7+0.9	3 (60.0)	1 (20.0)	-
41 – 45	3	0 (0.0)	0 (0.0)	-	-	-	-	-	-
Total	300	45 (15.0)	19 (6.3)	1421.1+748.8	2010+6534.7	10.2+1.8	21 (46.7)	16 (35.6)	2 (4.4)
		3.069	4.624	0.506	0.868	5.993	15.446	7.842	5.195
P-value		0.689	0.463	0.732	0.491	0.001	0.013	0.098	0.268

Grading of Anaemia by WHO (2008): Mild anaemia PCV 30-36%/Hb 10-11.9g/dl; Moderate anaemia PCV 21-30%/Hb 7-9.9g/dl; Severe anaemia PCV 18-21%/Hb 6.0-6.9g/dl

Table 4: The prevalence of malaria parasites infection, haemoglobin (Hb) levels and degree of anaemia by trimesters Test (Infected Subjects)

Age group (yrs)	No examined	No (%) with malaria parasite infections	No (%) with hookworm infection	Mean hookworm ova count±SD e/g	Mean parasite count±SD/µl of blood	Mean Hb±SD g/dl	No (%) with mild anaemia	No (%) with moderate anaemia	No (%) with severe anaemia
1st	39	3 (7.7)	2 (5.1)	2000.0+4243.0	694.5+970.3	9.2+1.1	1 (33.3)	2 (66.7)	0 (0.0)
2nd	171	22 (12.9)	10 (5.8)	1469+6811.6	1660.8+4119.4	10.1+19	12 (54.5)	6 (27.3)	2 (9.1)
3rd	90	20 (22.2)	7 (7.8)	1200.0+8813.9	2592.1+8903.0	10.6+1.7	20 (22.2)	8 (40.0)	0 (0.0)
Total	300	45 (15.0)	19 (6.3)	1421.1+788.8	2010.3+6534.7	10.2+1.8	21 (46.7)	16 (35.6)	2 (4.4)
		4.310	1.772	0.907	0.165	0.903	1.120	2.098	2.188
P-value		0.116	0.617	0.424	0.848	0.413	0.571	0.350	0.335

Grading of Anaemia by WHO (2008): Mild anaemia PCV 30-36%/Hb 10-11.9g/dl; Moderate anaemia PCV 21-30%/Hb 7-9.9g/dl; Severe anaemia PCV 18-21%/Hb 6.0-6.9g/dl

while the lowest prevalence of 694.5±970.3/µl of blood was observed amongst the subjects in their 1st trimester. The difference in distribution of mean parasite density was not statistically significant (X^2 =0.165, (df)=2, p=0.848). Pregnant women in their 3rd trimester had highest prevalence of 7(7.8%) while the subjects in their 1st trimester had the lowest prevalence of 2(5.1%). The difference in prevalence of hookworm infection was not statistically significant (X^2 =1.772, df=5, p=0.617). Subjects in their 1st trimester had the highest hookworm ova density of 2000.0±4243.0 e/g of stool while subjects in their 3rd trimester had the lowest worm burden of 1200.0±8813.9 e/g of stool. The difference in distribution of mean parasite density was not statistically significant (X^2=0.907, (df)=2, p=0.424). Pregnant women in their 3rd trimester had highest prevalence of 7(7.8%) while the subjects in their 1st trimester had the lowest prevalence of 2(5.1%). The difference in prevalence of hookworm infection was not statistically significant (X^2 =1.772, df=5, p=0.617). Subjects in their 1st trimester had the highest hookworm ova density of 2000.0±4243.0 e/g of stool while subjects in their 3rd trimester had the lowest worm burden of 1200.0±8813.9 e/g of stool. The difference in distribution of mean parasite density was not statistically significant (X^2=0.907, (df)=2, p=0.424). The highest mean Hb of 10.6±1.7g/dl occurred among the subjects in their 3rd trimester while the lowest mean Hb 9.2±1.1g/dl occurred among the subjects in their 1st trimester The difference in mean Hb levels was not statistically significant (X^2 =0903, (df)=2, p=0.413).Severe anaemia had the highest prevalence of 2(9.1%) among the subjects in their 2nd trimester while the lowest prevalence of 0(0.0) among the subjects in their 1st and 3rd trimesters. The difference was not statistically significant (X^2 =2.188, (df)=0, p=0.335). Table 5 shows the distribution of haemoglobin levels of study subjects with independent plasmodium parasitaemia, hookworm infection and co-infection. The highest prevalence of infection 45(15.0%) was among the malaria alone and the lowest prevalence of 9(3.0%) was among the both infections. The highest of mean Hb 10.5±1.4g/dl was among hookworm alone while the lowest mean Hb

of 8.1±2.1g/dl among those with co-infections. The difference in mean Hb level of co-infected was statistically significant ($F_{2,70}$=0413, p=0.003).

DISCUSSION

The result of this research work has revealed that 3.0% of the test subjects were co-infected with *Plasmodium* and hookworm. The prevalence observed in this study is lower than 13.0% observed by Ekejindu et al[13] in a semi-urban area of Anambra State, Nigeria. This lower prevalence of malaria and hookworm co-infection may be due to differences in the socio-economic status and hygienic levels of the study subjects. The conduct of the study in dissimilar areas of urban and sub-urban settlements could enhance the inconsistency of the findings. There was a higher prevalence of independent malaria infection (15.0%) than hookworm (6.3%). Women living in malaria endemic areas have an increased risk of malaria parasitemia during pregnancy.[14] Also there is a hypothesis that multicellular organism like hookworm causes polarization of host immune Response it infection without causing same to unicellular organism like *Plasmodium,* a protozoan.[15] This variation in stimulation of host immune response may be accountable for more increased prevalence of malaria than a hookworm.

Pregnant women in their second trimester of pregnancy had the highest prevalence of malaria and hookworm co-infection and the highest prevalence of severe anaemia 80.0%. From the results of this study, The prevalence of co-infection of malaria and hookworm is proportionate to the degree of anaemia in the study subjects. The analysis between malaria and hookworm positive (test) subjects and malaria and hookworm negative (control) subjects with the mean Hb shows that there was a significant difference statistically between the mean Hb among the malara infected women and the control subjects, but there is no significant difference in mean Hb levels among hookworm positive and control subjects.

Table 5: Distribution of Haemoglobin (Hb) levels of pregnant women with plasmodium Parasitaemia and hookworm infection (n = 300)

Pregnant subjects	No. (%) infected	Mean parasite count±SD/µl	Mean Hb±SD g/dl
Malaria	45 (15.0)	2010.3±6534.7	10.2±1.8
Hookworm	19 (6.3)	1421.1±748.8	10.5±1.4
Both infections	9 (3.0)	a. 725.9±10402.3	8.1±2.1
		b. 1188.9±666.0	
Total	73 (2.4)		10.9±6.6
		a) $F_{2,6}$=0.838	$F_{2,70}$=0.413
		P^a=0.477	P_1=0.003
		b) $F2,6$=3.675	P^2=0.004
		P^b=0.091	

Key: a) Mean malaria parasite count for both infections. b) Mean hookworm ova count for both infections

What has remained unclear is the extent to which hookworm and malaria is associated with anaemia during pregnancy. The result of this study shows that intensity of infection with hookworm and Malaria parasites is proportionate to the Hb levels. Layrisse and Roche[16] had similar findings in a study among school-aged children. Anaemia in developing countries like Nigeria has multiple causes, including micronutrient deficiencies, infectious diseases and inherited disorder.[17] Other factors that can also cause anaemia include, parity owing to repeated drain in iron stores, fetal demand and underlying maternal diseases, untreated malaria in early pregnancy[18,19] as such the observed relationship between haemoglobin and hookworm- malaria co-infection may be confounded by other causes of anaemia. In this study, anaemia was most prevalent in the third trimester of pregnancy than the second and third trimesters. The first trimester had the lowest prevalence. These results contradict the WHO[20] report that anaemia was more prevalence in the first trimester than in the second and third trimesters in Sub-Saharan African.

The definition of what constitutes anaemia in pregnancy has been the subject of lively debate for several years. According to WHO,[20] the normal haematocrit values and haemoglobin levels for classifying anaemia in pregnancy lies between 33% and 36% and 11g/dl and 12g/dl respectively. Also WHO[11] categorized the degree of anaemia using the PCV/Hb values of mild anaemia 30%-36%/10-11.9g/dl; moderate anaemia 21%-30%/7-9.9g/dl; severe anaemia 18-21%/6.0-6.9g/dl.

Malaria and anaemia differed between age group in this study. This might be due to host or environmental factors; it might also be attributed to age related immunity as a result of previous exposure to malaria in child-bearing ages.[14] The higher anaemia prevalence observed among the subjects in this study contradicts a finding in Ghana which indicated a lower prevalence of anaemia.[21] Pregnant women with successive births are believed to have had exposure to a variety of strains of malaria parasites and hookworm ova thereby developing somehow efficient immunity against most strains of the parasites.[22, 23] A study in Iran found similar results.[24] However, Glover-Amengor and his colleagues in their study reported lower prevalence of anaemia which was strongly associated with increasing parity.[21]

Many of the respondents in this study registered for antenatal care in their second and third trimester. This seems to be a common practice in Africa.[25] In a study conducted in Zaire, most of the pregnant women attended antenatal clinic (ANC) for the first time in their sixth or seventh month of gestation and made three to four visits before delivery.[25] Also in Nigeria Mockenhaupt et al[26] Had a similar observation. This practice is detrimental as it does not allow for early detection and correction of pregnancy associated complications such as anaemia. It is therefore very important to educate women on the need to register early for antenatal care.

The current findings revealed lower prevalence of intestinal nematodes, especially hookworm among pregnant women. An earlier study by Shulman et al,[27] reported an overall prevalence of 25.7% hookworm in pregnant women which is higher compared To that obtained in this study (9.3%). Even though it is clear that there is significant association between anaemia and hookworm infection, the outcome of this study however indicated otherwise.

The only *plasmodium* species detected among the pregnant women in this research was *P. falciparum*, the species predominantly found in the tropical and subtropical regions of Africa.[5] *Plasmodium falciparum* is also the most widespread species, accounting for up to 80.0% of malaria cases worldwide.[5]

In the present study, 15.0% prevalence of malaria parasitaemia in pregnant women was lower compared with 23.0% reported in Mozambique[28] and 26.8% reported in Malawi.[29] The prevalence of malaria in the present study among pregnant women was much lower than 47.5% reported in Onitsha, Nigeria, 42.0% reported in Ghana,[30] 57.5% reported in Gabon,[14] and 41.0% observed in Uyo, Nigeria.[31] The low rate of malaria prevalence observed in the present study could be due to better awareness and improved preventive measures.

It has been recognized that a temperature range of 20°C - 30°C and relative humidity of 60.0% and above were suitable for malaria parasite transmission.[32] Delay in starting pre-natal care during pregnancy may also have contributed to this prevalence. In addition, the transient depression of cell-mediated immunity in pregnancy that allows foetal allograft retention but also interferes with resistance to various infectious diseases such as malaria could be a contributing factor. The prevalence of degree of anaemia observed in this study (42.6%) was lower than the mean percentage for Africa which is 61.0%.[14,18,33] Any strategy aimed at combating this important public health challenge should aim at addressing all the potential causal factors after their elucidation.

The relatively low prevalence rate of 9.3% hookworm infections in this studied population could also be indicative of good sanitation and proper sewage disposal, as shown by the fact that the participants may had good toilet facilities in their respective homes. Findings from a city of Thailand

study disclosed an increase in the prevalence of clinical malaria in individuals with helminthes like hookworm infections.[34] Awareness of the importance of co-infections is increasing, and suggestions have been made that helminth infection may influence susceptibility to other infections, including malaria.[35, 36] Several hypotheses have been put forth to explain this observation. It has been suggested that helminthes infection creates a cytokine milieu favorable to the production of non cytophilic antibodies, thus making individuals more susceptible to clinical malaria.[37] It is also thought that the presence of T-regulatory cells is amplified during helminthes infection, and if present in sufficient numbers, could induce a non-specific suppression,[38] making individuals susceptible to infections such as malaria.

Hookworms shed eggs intermittently according studies; therefore, the prevalence of this infection is likely to be underestimated in this study. This study shows relatively low prevalence rates of malaria and hookworm co-infection in pregnant women in University of Calabar Teaching Hospital. Other intestinal parasites observed and recorded in this study alongside with hookworm include *Ascaris lumbricoides, Entamoeba histolytica,* and *Taenia species*. But they played no impact in this study because they were not the objective of this research.

CONCLUSION

The work has shown a low prevalence of malaria and hookworm Co-infection among pregnant women in Caalabar. The degree of anaemia among pregnant women who had malaria and hookworm co-infection was more severe than those that had independent malaria infection and independent hookworm infection and subjects without any of the infection. Also the prevalence of malaria infection/density and hookworm infection increases from first trimester to the third trimester. It is therefore recommended that there is the need to put in place necessary strategies to control these infections amongst this category of subjects.

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REFERENCES

1. Hartman J and Feldmeier H. Epidemiological and Clinical characteristics of hookworm-related cuteneous larva migrans. Lancet Infect Dis 2012; 8(5):302-309.

2. Brooker S, Bundy DAP, Cook GC and Zumla AI. Soil-transmitted Helminths (Geohelminths). eds. Manson's Tropical Diseases. 22nd ed. Philadelphia, PA: WB Sanders. 2009; 1515-1548.

3. World Health Organisation. The prevalence of anaemia in women: a tribulation of available information. Geneva, Switzerland 1992; WHO/MCH/MSH/92.2.

4. Croese J, Loukas A, Opdebeeck J, Failey S and Prociv P. Human Enteric 2012; 45(2): 265-271.

5. Cheersbrough M. District Laboratory Practice in Tropical Countries Cambridge. University Press, Cambridge 2005; (6): 145-156.

6. Brooker S, Bethony J and Hotez PJ. Human hookworm infection in the 21st century. Advance Parasitology 2004; 58: 197-288.

7. Mbipom AK, Neema S and Magnussen P. "Preventing Malaria in Pregnancy: A study of Perceptions and Policy Implications in Mukono District, Uganda" Health Policy and Planning 1996; 21(1): 17-26.

8. United Nation. Department of Economic and Social Affair, Population Division.2014; 6-12

9. World Health Organization (WHO). Fact sheet No. 94. W.H.O., Media Centre, 2010. Available from http://whqlibdoc.who.int/publications/2010/9789241547826_eng.pdf.

10. World Health Organisation. Parasitic Diseases. 2012 Available at http://www.who.int/vaccineresearch/diseases/parasitic.

11. World Health Organization. Iron deficiency anaemia – Assessment, prevention and control. A guide for programme managers. Geneva: World Health Organisation. 2008; 6-8.

12. Stoll NR. On endemic hookworm, where do we stand today? Exp Parasitol 1962; 12:241-252.

13. Ekejindu TM, Okeke EK, Akah B, Okpala E, Ezeagwuna DA and Onwurah P. Malaria and hookworm co-infection among pregnant and non-pregnant women in a semi-urban areas in Anambra State. World J Med Sc 2011; 6(1): 33-35.

14. Bouyou-Akotet MK, Lonete-Collard DE, Mabika-Manfoumbi M, Kendjo E, Matsiegui PB, Mavougou E, et al. Prevalence of Plasmodium falciparum infection in Pregnant women in Gabon. J Malaria 2003; 2(1): 18.

15. Maizels L, Tribolet L and Simon A. Probing the equatorial groove of the hookworm protein and vaccine candidate antigen, Na-ASP-2. Intern J Biochem and Cell Biol 2004; 50:146-155.

16. Layrisse M and Roche M. The relationship between anaemia and hookworm infection. Results of surveys of rural Venezuelan population. Am J Hyg1964; 79:279-301.

17. Tolentino K and Friedman JF. An update on anaemia in less developed countries. Am J Trop Med and Hyg 2007; 77:44-51.

18. Van den Broek NR, Rogerson SA, Mhango CG, Kambala B, White SA and Molyneux ME. Anaemia in pregnancy in Southern Malawi: Prevalence and risk factors. Brit J Obst and Gynae. 2000; 107, 445-451.

19. Bukar M, Audu BM, Yahaya UR and Melah GS. Anaemia in pregnancy at booking in Gombe, North-Easthern Nigeria. J Obst and Gynae 2008; 28:775-778.

20. WHO. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System. Geneva, World Health Organization, 2011 (WHO/NMH/NHD/MNM/11.1)(http://www.who.int/vmnis/indicators/haemoglobin.pdf, accessed 3rd April, 2018).

21. Glover-Amengor M, Owusu WB and Akanmori B. Determinants of Anaemia in pregnancy in Sekyere West District, Ghana" Ghana Med J 2005; 39(3): 102-107.

22. Beeson JG, Rogerson SJ, Cooke BM, Reeder JC, Chai W, Lawson AM, et al. Adhesion of *Plasmodium falciparum*-infected Erythrocytes to Hyaluronic Acid in Placental Malaria Nature Med 2000; 6(1): 86-90.

23. Fried M and Duffy PE. Adherence of Plasmodium falciparum

to Chondroitin Sulfate A in the Human Placenta. Science 1998; 272(5267): 1502-1504.

24. Veghari GR, Mansourian R and Marjani AJ. The Comparison of the anaemia in pregnant and non-pregnant women in the villages of the South-East of Caspin Sea-Gorgan-Iran. J Med Sc 2007; 7(2): 303-306.

25. Okonofua FE and Abejide OR. "Prevalence of Malaria Parasitaemia in Pregnancy in Nigerian Women," J Obst & Gynae 1996; 16(5): 311-315.

26. Mockenhaupt FB, Rong B, Gunther M, Beck S, Till H, Kohne E, et al. Anaemia in pregnant Ghanaian women: Importance of Malaria, Iron Deficiency 2000; 57: 2-8.

27. Shulman CE, Dorman EK, Cutts F, Kawuondo K, Bulmer JN, Peshu N, et al. Intermittent Sulphadoxine-Pyrimethamine to prevent severe anaemia secondary to malaria in pregnancy: A randomised placebo controlled trail. The Lancet 1999; 353(9153): 632-636.

28. Saute F, Menendez E, Mayor KA, Aponte J, Gomez-Olive X, Dgedge M, et al. Malaria in pregnancy in rural Mozambique: A role of Parity, Submicroscopic and Multiple Plasmodium falciparum infections. Trop Med Intern Health 2002; 7(1): 19-28.

29. Rogerson SJ, van den Broek NR, Chaluluka E, Qongwane C, Mhango CG and Molyneux ME. Malaria and Anaemia in Antenatal Women in Blantyre, Malawi: A Twelve-Month Survey. Am J Trop Med & Hyg 2000; 62(3): 335-340.

30. Ouma P, Hamel MJ, Parise M, Ayisi JG, Otieno K, Kager PA, et al. Malaria and Anaemia among Pregnant Women at First Antenatal Clinic visit in Kisumu, Western Kenya. Trop Med & Intern Health 2007; 12(12): 1515-1523.

31. Opara KN, Ibanga ES, Wali NB and Usip LP. Falciparum Malaria and Susceptibility to Genetic Markers of Pregnant Women in Uyo, Southeast Nigeria," Abstract of the 28th Annual Conference of the Nigeria Society of Parasitology held at Imo State University Owerri in 2004. 76.

32. World Health Organisation. "WHO Expert Committee on Malaria. 2000 Twentieth Report.

33. Nail S and Nail AS. Effects of Malaria infection on Pregnancy. Indian J Malariol 2000; 30(4): 207-214.

34. Nacher M, Singhasivanon P, Yimsamran S, Manibuynong W, Thanyavanich N, Wuthisen P, et al. Intestinal helminth infections are associated with increased incidence of Plasmodium falciparum malaria in Thailand. J Parasitol 2002; 88:55-58.

35. Bentwich Z, Maartens G, Torten D, Lal A and Lal R. Current infections and HIV pathogenesis. AIDS 2000; 14: 2071-2081.

36. Nacher M. Malaria vaccine trails in a wormy world. Trends Parasitol 2001; 17: 563-565.

37. Mwangi TW, Bethony J and Brooker S. Malaria and helminth interaction in humans: an epidemiological viewpoint. Annals Trop Med Parasitol 2006; 100: 551-570.

38. Yazdanbakhsh M, van der Biggelaar A and Maizels RM. Th2 responses without atopy: immunoregulation in chronic helminth infections and reduced allergic disease. Trends Immunol 2001; 22: 373-377.

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