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The Prevalence of Malaria and Helminth Infection in Pregnancy at Booking and Their Relationship to Anaemia at the University of Port Harcourt Teaching Hospital, Southern Nigeria

E. W. Nnah¹ and T. Kasso^{2*}

¹Department of Obstetrics and Gynaecology, University of Port Harcourt Teaching Hospital (UPTH), Port Harcourt, Nigeria. ²Department of Obstetrics and Gynaecology, University of Port Harcourt, Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author EWN designed the study, wrote the protocol and the first draft of the manuscript. Author TK performed the statistical analysis and did the literature searches. Authors EWN and TK managed the analyses of the study. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Objectives: To determine the prevalence of malaria and helminth infection in pregnancy and their relationship with anaemia at booking at the University of Port Harcourt Teaching Hospital. **Materials and Methods:** This was a cross-sectional descriptive study of 192 pregnant women who booked for antenatal care at the University of Port Harcourt Teaching Hospital between August 1, 2015, and March 31, 2016. Socio-demographic data were collected through a structured questionnaire. Blood samples were collected and thick and thin blood films made, stained and examined for malaria parasites under a light microscope using x100 objective lens with oil immersion. Wet mount was prepared from the stool specimen using direct smear method with

*Corresponding author: Email: terhemen.kasso@uniport.edu.ng;

normal saline and iodine preparation, and the concentration procedure using formol/ether for the identification of ova of helminths. Data obtained was analysed using Statistical Package for Social Sciences (SPSS) version 20.0. The results are presented in frequency tables and figures.

Results: The prevalence of malaria, helminth, and their co-infection at booking were 24.5%, 0.5% and 0.5% respectively while the prevalence of anaemia at booking was 16.7%. Malaria and helminth co-infection accounted for 3.1% of the study population with anaemia while 75% of those with anaemia had malaria infection alone. The helminth infection identified in this study was Ascaris lumbricoides. There was a statistically significant relationship between malaria and helminth co-infection, and the area of residence (p = 0.036).

Conclusion: The prevalence of malaria and helminth co-infection was very low and had no statistically significant relationship with anaemia. Malaria infection was mainly associated with anaemia at booking.

Keywords: Pregnancy; malaria; helminth; co-infection; Southern Nigeria.

1. INTRODUCTION

Parasitic infection during pregnancy is a major cause of anaemia in Africa [1]. Malaria and helminthiases are co-endemic especially in sub-Saharan Africa, and there is an extensive overlap between the area of malaria and helminth infection in Africa [1,2]. Their co-infection is not uncommon, and its additive effect may lead to worsening anaemia and other morbidities, this could have dire consequences on pregnancy [1,2].

According to the World Health Organization (WHO) estimate, there were 212 million new cases of malaria worldwide in 2015 (148-304million), with Africa contributing 90% of the global cases of malaria. About 429.000 (235.000 639,000) deaths occurred from malaria worldwide, most of these deaths occurred in Africa (92%) [3]. Nigeria is one of the most endemic countries for malaria, and the susceptibility to this infection is increased by pregnancy [4]. Malaria infection in pregnancy is a major health challenge as it poses a substantial risk to the mother, her fetus and the neonate. Many studies have demonstrated the relationship between malaria in pregnancy and maternal anaemia, miscarriage, placental parasitaemia, low birth weight, congenital malaria, stillbirth, and maternal death depending on the level of transmission [5-8].

Helminths are parasitic worms. Helminth infections are more common in the developing countries and produce a global burden of disease greater than that of other known infectious diseases such malaria as or estimated tuberculosis [9]. lt is that approximately one-third of the 3 billion people in the developing region of Sub Saharan Africa, Asia and the Americas that live on less than 2 US dollar per day are infected with one or more helminths [1,10]. Helminth infection during pregnancy is associated with anaemia. prematurity, low birth weight, and increased mortality maternal morbidity and [11]. Haemoglobin concentration of <11 g/dl or PCV < 33% is considered as anaemia in pregnancy by WHO [12].

Co-infection of *P. falciparum* and hookworm has been reported to have an additive impact on haemoglobin by increasing the susceptibility to *P. falciparum*, which could have a great consequence on the pregnancy outcome because it results in intrauterine growth restriction, low birth weight, pre-term delivery and neonatal mortality [13]. The adverse perinatal outcomes may include premature, hypotrophic neonates and stillbirths in the malarial population, which may be linked to intervillous macrophages that decrease the maternal blood output and the perivillous excess of fibrin that reduces the materno-fetal exchanges [14].

Studies on the co-infection of *P. falciparum* and intestinal parasites among the pregnant women in Southern Nigeria are lacking. This study was conducted to determine the prevalence of malaria and helminth infection in pregnancy at booking and their relationship to anaemia.

2. MATERIALS AND METHODS

2.1 Study Site

The study was done at the ante-natal clinic of the University of Port Harcourt Teaching Hospital. The hospital is located in Alakahia town in Obio Akpor Local Government Area of Rivers State. It is the apex health institution in Port Harcourt and provides specialized healthcare services for Port Harcourt and the entire Rivers State, as well as neighboring states. The antenatal clinic runs every day from Monday to Friday headed by Consultant Obstetricians on each day. An average of 5000 antenatal women is seen annually, which amounts to about 400 women monthly with about 3000 admissions.

The prevalence rates of malaria parasite infection among pregnant women in the area were 27%, 27.3%, and 21% in the first, second and third trimesters respectively [15]. Malaria control in pregnant women in the area is with the use of insecticide-treated nets (ITNs) and Intermittent Preventive Treatment in Pregnancy (IPTp) with sulphadoxie/pyrimethamine.

2.2 Sample Size Calculation

The sample size for this study was calculated using the formula for cross-sectional studies

 $n = (Z^2 P (1-P)/d^2) [16]$

Where

n = sample size

Z = proportion of normal distribution corresponding to the required significance level (5%) which is 1.96

P = prevalence of malaria hookworm co-infection which is 13% [17]

d = degree of accuracy or precision expected (0.05)

Thus $n = 1.96^2 \times 0.13 (1-0.13) / 0.05^2 = 173.79 = 174$

The sample size was thus calculated to be 174. Giving an allowance for 10% attrition rate, the minimum sample size for the study was therefore 191 women.

2.3 Methods

This cross-sectional descriptive study was conducted among pregnant women booking for ante-natal care at the University of Port Harcourt Teaching Hospital from August 1, 2015, to March 31, 2016. The sample size for this study was calculated using the formula for cross-sectional studies, using a previous prevalence of malaria hookworm co-infection which was 13% [17].

Pregnant women who had antimalarial drugs either as chemoprophylaxis or for treatment prior to booking, those who had anti-helminthics prior to booking, those with Human immunodeficiency virus infection (HIV), and pregnant women with sickle cell disease and other haemoglobinopathies were all excluded from the study.

All pregnant women who satisfied the criteria and gave consent were recruited for the study. Sociodemographic data were collected using a structured questionnaire and included age, level of education, occupation, marital status, area of residence, religion and parity. The questionnaires were administered by the principal investigator and some trained assistants.

2.4 Blood and Stool Sample Collection

Blood film was made on a clean slide by placing a drop of blood on the slide and spreading it to an area of approximately 1cm². Safety procedure was followed in the collection of venous blood sample by swabbing the antecubital fossae with 70% alcohol and five milliliters of blood was drawn into an ethylene diamine tetra-acetic acid (EDTA) bottle with a sterile hypodermic needle. The thick film was air dried and the slide was dipped into Field's stain A for 3 seconds. The slide was then dipped into tap water for 3 seconds and then Field's stain B for another 3 seconds. It was washed gently with tap water for a few seconds until the excess stain was removed and allowed to dry vertically. The same procedure was used for the thin film except that Field's stain B was applied first. Thin film was used to identify parasite species which was P. falciparum while thick film was used for parasite density. Malaria parasite density was done using the number of parasites per high power field (hpf) and classified as 1+ (1-9 parasites 00 hpf), 2+ (≥ 10 parasites 00 hpf), 3+ (1-9 parasites/hpf), $4+ (\geq 10 \text{ parasites/hpf})$ [18].

2.5 Microscopic Examination

The thick film was used to determine the parasite densities. Stained slides were examined under the light microscope using X100 objective lens in oil immersion.

Non-heparinized capillary tubes were filled with a blood sample from the EDTA bottle. The tips of the capillary tubes were cleaned with cotton wool, sealed and arranged inside a haematocrit centrifuge. They were centrifuged for 5 minutes at 12,000 revolutions per minute. The Packed Cell Volume was determined by using a haematocrit reader. Aneamia was graded using

WHO haemoglobin values for anaemia in pregnancy as 10.0 - 10.9g/dl (mild), 7 – 9.9 g/dl (moderate), <7 g/dl (severe) [19].

All patients were given clean labeled stool containers and instructed to bring small quantities of their stool specimen on their next visit within an hour of passing the stool to the laboratory. Wet mount was prepared from the stool specimen using direct smear method with normal saline and iodine preparation, and the concentration procedure using formol-ether for the identification of helminth eggs as well as the protozoan cyst.

2.6 Statistical Analysis

Statistical package for Social Sciences SPSS version 20 was used for data analysis. The results are presented in means, standard deviation and frequency tables. Chi square was used to determine the correlation between variables. Statistical significance was reported at P<0.05.

3. RESULTS

Of the 192 women recruited into the study, 160 (83.3%) were aged between 18-35 years, with a mean age at booking of 30.52 ± 4.75 years. The modal parity was para 1-4 with 53.1% of the study population in this group. Most (87%) of the women had tertiary education and majority (96.4%) of them resided in urban area while 3.6% resided in semi-urban area. Almost all (98.4%) of the women were Christians. These are shown in Table 1.

The prevalence of malaria infection was 24.5%. Majority (89.4%) had mild parasite density and 10.6 % had moderate parasite density while that of helminth infection was 0.5%, and the infection was of moderate parasite density. The age, marital status, educational level and other variable had no significant association with the prevalence of helminth infection at booking. The prevalence of malaria and helminth co-infection in the study population was 0.5%. The mean Packed Cell Volume was 32.67 ± 3.33 %. The prevalence of anaemia at booking was 16.7%. Majority, 28 (87.5%) of the study population with anaemia had mild anaemia while 12.5% (4) had moderate anaemia. These are all shown in Table 2.

Those who resided in semi-urban areas had the highest prevalence of malaria infection at 42.9%, however this was not statistically significant

(p>0.05). The prevalence of malaria by parity was 29.3% in para 0, 21.6% in para 1-4, and 12.5% in \geq para 5. The distribution of malaria parasitaemia among other co variates were as shown in Fig. 1.

Of the 192 women, only 1 had malaria and helminth co-infection and the helminth identified was Ascaris lumbricoides. The malaria parasite and helminth infection density were both moderate in this participant who resided in a semi-urban area. There was a statistically significant relationship between malaria and helminth co-infection, and the area of residence (p= 0.036). Other variables such as age, parity, marital status, level of education and religion had no statistically significant relationship with malaria and helminth co-infection (p>0.05). Fig. 2 shows the results of malaria and helminth infection prevalence.

Most (71.4%) of the women with mild anaemia tested positive for malaria parasite while 28.6% (8) with mild anaemia were negative for the malaria parasite. All the women with moderate anaemia tested positive for malaria parasite as shown in Fig. 3. There was a very low negative correlation (r= -0.13) between malaria and helminth co-infection, and anaemia in this study.

Table 1. Socio-demographic characteristics

Variable	Number	Percentages
Age (years)		
<18	0	0%
18-35	160	83.3%
>36	32	16.7%
Marrital status		
Married	192	100%
Single	0	0%
Divorced	0	0%
Separated	0	0%
Level of education		
Tertiary	167	87.0%
Secondary	24	12.5%
Primary	1	0.5%
Parity		
0	82	42.7%
1-4	102	53.1%
>4	8	4.2%
Area of residence		
Urban	185	96.4%
Semi-urban	7	3.6%
Rural	0	0%
Religion		
Christianity	189	98.4%
Islam	3	1.6%
Others	0	0%

Variable	Frequency	Percentage
Results for malaria infection		
Positive for malaria parasite		
No	145	75.5%
Yes	47	24.5%
Parasite density		
+	42	89.4%
++	5	10.6%
+++	0	0%
Results for helminth infection		
Stool microscopy positive		
No	191	99.5%
Yes	1	0.5%
Density of infection		
Mild	0	0%
Moderate	1	100%
Severe	0	0%
Malaria helminth co-infection		
No	191	99.5%
Yes	1	0.5%
Haematological result		
Normal PCV	160	83.3%
Anaemia	32	16.7%
Degree of anaemia		
Mild	28	87.5%
Moderate	4	12.5%
Severe	0	0%

Table 2. Laboratory results



Fig. 1. Distribution of malaria parasite infection among variables

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Fig. 2. Malaria and helminth infection prevalence

Fig. 3. Degree of anaemia in relation to malaria infection

4. DISCUSSION

The findings from this study suggest that pregnant women were more susceptible to malaria than helminth infection at booking at the University of Port Harcourt Teaching Hospital. This was similar to reports from previous studies [20,21]. The prevalence of malaria was similar to that obtained in Gabon [21]. This may be because the study population was similar, although the sample size of this study was approximately half that of the one conducted in

Gabon. It was however lower than that reported in some other studies [17,20,22]. The higher prevalence in these studies could be as a result of the study location, since all the study participants resided in semi-urban areas with a lot of bushy surroundings, poor sanitation and waste disposal facility while the majority of the participants in our study resided in urban areas. In this study, those residing in semi-urban areas had the highest prevalence of malaria compared to those who resided in urban areas. It can therefore be deduced that residence in a semiurban area was associated with a higher prevalence of malaria. The study further reaffirms a higher prevalence of malaria infection among primigravidae when compared to multigravidae which were also reported in earlier studies [17,21,22].

The prevalence of helminth infection in this study was 0.5%, which was very low when compared to reports from other studies [17,21,23]. This very high prevalence can be attributed to the methodology used in those studies, the sample population and their area of residence. In the study done in Gabon, stool samples were tested a minimum of three times for helminth infection at monthly interval if the initial stool sample tested was negative unlike in this study where the stool samples of participants were tested only once. The high prevalence of helminth infection as reported in Enugu and Anambra when compared to this study could be attributed to the fact that the studies were done in semi-urban and rural areas respectively, which were surrounded by thick forest with no modern toilet and water drainage facilities. In contrast, the majority of participants in this study were well educated and resided in an urban area with modern toilet and water drainage facilities, which may have accounted for the low prevalence of helminth infection in the study.

Malaria and helminth co-infection in this study were rather low when compared to previous reports [17,20-22]. The high prevalence of malaria and helminth co-infection in these studies could be attributed to the methodology used, socio-demographic variables such as residence in a rural area or semi-urban area. parity and low socio-economic status Participants in this study resided mainly in an urban area and were highly educated, which could account for the low prevalence of malaria and helminth co-infection. However, there was a statistically significant relationship between malaria and helminth co-infection and area of residence, which showed an association between those residing in semi-urban area and malaria and helminth co-infection.

The prevalence of anaemia in this study was lower than the findings from other reports [17,23,24]. However, there was no statistically significant association between malaria and helminth co-infection with anaemia. Majority of the women with anaemia had malaria, this suggested that malaria infection was a more common cause of anaemia than helminth infection or their co-infection in our center. A similar finding was also reported in other studies [24,25].

5. LIMITATION

This study was carried out at a tertiary center located in an urban area, therefore it may not reflect the true picture of malaria and helminth co-infection and their effect on anaemia in the rural communities of Rivers State.

6. CONCLUSION

There was a very low prevalence of malaria and helminth co-infection at booking at the University of Port Harcourt Teaching Hospital. Larger studies and rural community-based studies are warranted.

CONSENT

A written informed consent was given by the patients to participate in the study and a verbal informed consent was obtained from them for the publication of this work.

ETHICAL APPROVAL

The authors hereby declare that the research was approved by the hospital ethics committee. The ethical approval protocol number is UPTH/ADM/90/S.II/VOL.X/500.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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