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Article in *Asian Pacific Journal of Tropical Disease* · June 2016

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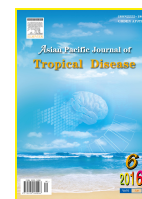
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journal homepage: www.elsevier.com/locate/apjtd

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Pattern of *Plasmodium*-intestinal helminth co-infection among pregnant women in a high transmission zone of malaria in Nigeria

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ARTICLE INFO

Article history:

Received 17 May 2016

Accepted 26 May 2016

Available online 21 Jun 2016

Keywords:

Malaria-helminths
Pregnancy
Anaemia
Ilorin
Aggravate
Hygiene

ABSTRACT

Objective: To investigate the co-infection of malaria and intestinal helminths and its burden among the pregnant women in Kwara State, Nigeria.

Methods: Blood and faecal samples of pregnant women were randomly examined using blood smear and Kato-Katz techniques, respectively. Micro-haematocrit reader was used to estimate packed cell volume (PCV) while information concerning pregnancy and transmission factors was obtained by questionnaire.

Results: Out of the 300 pregnant women, 17.3% had at least one parasite infection and the specific rate of co-infection was 73.1%. Co-infection of *Plasmodium falciparum* and *Ascaris lumbricoides* decreased with increasing age while the concurrence of *Plasmodium falciparum* with hookworm increased with increasing age of women. It was observed that helminth infection protected the severity of malaria and aggravated anaemia level. Pregnant women with malaria alone had average parasitaemia of 1034.9 parasite/μL of blood and PCV of 30.24% while individuals co-infected with hookworms had parasitaemia of 859.67 parasite/μL and PCV of 26.98%. Our findings also indicated that the prevalence of infection in pregnancy varied with gestation periods. The highest prevalence was recorded in pregnant women in their primigravidae and first trimester. Inadequate toilet facilities, illiteracy, occupations, low incomes and proximities of vegetation around the habitation were observed to influence the transmission of multiple parasites.

Conclusions: Mass drug administration and maintenance of personal and environmental hygiene are essential preventive measures in endemic communities to ward off the debilitating effects of parasites in pregnancy.

1. Introduction

Pregnancy-associated malaria results in substantial maternal and fetal morbidity, causing 75 000–200 000 infant deaths every year. Pregnant women are more susceptible to malaria than non-pregnant women, and the susceptibility is highest in primigravidae

even in low transmission area[1,2]. The susceptibility to malaria during pregnancy has not only been attributed to immunological and hormonal changes associated with pregnancy but also with the unique ability of a subset of infected erythrocytes to sequester in the placenta[3,4].

Extensive evidences confirm the importance of protective antibodies directed against the surface of infected erythrocytes in the placenta of multigravidae, which is usually absent in first pregnancy, leading to adverse pregnancy outcomes, including maternal anaemia, low birthweight, fetal growth restriction splenomegaly and congenital transmission[5].

In sub-Saharan Africa, there exists a broad geographic overlap between *Plasmodium falciparum* (*P. falciparum*) and an intestinal helminth infection due to favorable environmental conditions

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The study protocol was performed according to Helsinki declaration and approved by Kwara state ministry of health and University of Ilorin ethical research committee. Informed written consent was obtained from Chief Medical Officers of each health centres and the consented individual

Foundation Project: Supported by University of Ilorin Senate Research (Grant No. UIIL/REF/2014/5370).

The journal implements double-blind peer review practiced by specially invited international editorial board members.

and human behavioral activities that enhance the survival and transmission of the parasites[6,7]. The effects of multiple parasite infections in a host have serious public health implications, many of which remain unknown[8]. For instance, existing data suggest that the co-infection of *P. falciparum* and hookworm has an additive impact on hemoglobin; exacerbating anemia-related malaria disease burden in pregnancy[6]. This is a common problem in many developing countries where poverty, ignorance and diseases are more prevalent. It is globally estimated that 58.27 million women are anaemic during pregnancy, and 55.75 million (95.7%) live in developing countries[9].

In Nigeria, epidemiological data on concomitant parasitic infections in pregnant women are largely limited. For effective planning and control programs, appropriate comprehensive data are necessary. We therefore investigate the co-infection of malaria and intestinal helminths and its burden among the pregnant women in Kwara State, Nigeria.

2. Materials and methods

2.1. Description of study areas

The study was conducted among pregnant women attending antenatal care in two health centres (Sobi Specialist Hospital and Ajikobi Cottage Hospital) in Ilorin. Ilorin is located between longitude 08°29'21" E, latitude 04°30'50" N and longitude 08°29'43" E, latitude 04°31'01" N. It has typical tropical climate with well-defined wet (April–October) and dry (November–March) seasons. Inhabitants are predominantly subsistence farmers, with few petty traders and civil servants. The community is mainly made up of Yoruba and Fulani ethnic groups although a small proportion of the people belong to Hausa, Nupe and Ebira ethnic groups. The sanitary condition in many areas of the community is precarious as human and domestic wastes are littered around human habitations and water drainage system is poor. Most of the houses lack protective mosquito nets.

2.2. Data and sample collection

A hospital-based study was conducted on pregnant women between January and June, 2015. After the collection of informed consent forms from volunteers, a pre-tested structured questionnaire was administered to access the bio-demographic and epidemiological factors of the participants. Women were interviewed by one of the investigators (Olarewaju Abdulkareem Babamale) on the possible risk factors. Thereafter, stool and blood samples of each subject were collected in a pre-labelled specimen container and ethylene diamine tetraacetic acid bottle respectively with detailed instruction on hygienic handling of samples. Samples were transported to University of Ilorin Teaching Hospital, Ilorin, Nigeria where parasitological examinations were conducted using blood film and Kato-Katz thick smear techniques

for malaria and intestinal parasites, respectively. Only participants that were positive for malaria were examined for intestinal helminths. The number of parasites per microliter of blood was calculated using Greenwood and Armstrong[10] and stool samples were prepared under microscopy within 1 h after collection. The capillary technique using Hewkley micro-haematocrit reader was used for the estimation of the packed cell volume (PCV).

2.3. Ethical consideration

The study protocol was performed according to the Helsinki Declaration and approved by Kwara State Ministry of Health and University of Ilorin Ethical Research Committee. The informed written consent was obtained from chief medical officers of each health centres and the consented individuals. Participants who were positive for any of the parasites were referred for treatment in the hospital.

2.4. Statistical analysis

All analysis was performed using SPSS version 16.0 for Windows (SPSS Inc. Chicago, IL, USA). Differences in the prevalence and intensity of infections between ages and sexes were tested using the *Chi-square* and One-way ANOVA tests, respectively. Values are considered statistically significant when *P* values were less than 0.05.

3. Results

Out of the 300 pregnant women examined, 52 (17.3%) were positive for at least one parasite species. Overall, 12.7% of our study population recorded multiple parasitic infections. The prevalence of infections according to age groups was comparable but not significant ($P > 0.05$) except for the co-infection of malaria and hookworm ($P = 0.048$). The co-infection of *P. falciparum* and *Ascaris lumbricoides* (*A. lumbricoides*) decreased with increasing age while the concurrence of *P. falciparum* with hookworm increased with increasing age. The infection status with respect to gravidae and parity of the pregnancy revealed that individuals in primigravidae and first trimester showed a significant high prevalence of malaria and intestinal helminths (Table 1).

The infection pattern with respect to PCV and parasitaemia level of *P. falciparum* is similar. The average PCV in uninfected pregnant women was observed to be 40.03% while individuals with malaria alone, malaria + hookworm and malaria with 2 helminth parasites (*A. lumbricoides* + hookworm) recorded an average PCV of 30.24%, 26.98% and 25.32% respectively. Similar trend in PCV was observed with decreasing levels of parasitaemia (Table 2). Analysis of socio-economic and environmental factors of the pregnant women revealed that factors such as lack of toilet facilities, illiteracy, occupations and low/no monthly incomes were the overlapping factors that influenced multiple parasites

in infected individuals (Table 3). Further analysis that infected pregnant that sleeps outside in environments without net recorded high prevalence of malaria infections. The presence and proximity of bushes around the habitation predisposed pregnant women to high occurrence of multiple infections (Table 3).

Table 1

Prevalence of *P. falciparum* and its co-infection stratified by age and gestation period of pregnancy.

Variable		Examined number	Malaria [<i>n</i> (%)]	Co-infection with intestinal helminths [<i>n</i> (%)]		
			<i>P. falciparum</i>	<i>A. lumbricoides</i>	Hookworm	<i>A. lumbricoides</i> + hookworm
Overall		300	52 (17.3)	15 (5.0)	11 (3.6)	12 (4.0)
Age group	15–21	28	9 (32.1)	5 (17.9)	2 (7.1)	0 (0.0)
	22–27	130	20 (15.4)	3 (2.3)	1 (0.8)	3 (2.3)
	28–33	101	18 (17.8)	4 (4.0)	3 (3.0)	5 (4.9)
	≥ 34	41	5 (12.2)	3 (7.3)	5 (12.2)	4 (9.8)
<i>P</i> value			0.092	0.065	0.048	0.195
Gravidity difference	Primigravidae	95	27 (28.4)	7 (7.4)	4 (4.2)	4 (4.2)
	Secondgravidae	90	16 (17.8)	3 (3.3)	2 (2.2)	5 (5.5)
	Multigravidae	115	9 (7.8)	5 (4.3)	5 (4.3)	3 (2.6)
<i>P</i> value			0.038	0.078	0.151	0.210
Parity of the pregnancy	First trimester (1–3 months)	32	10 (31.3)	6 (18.8)	1 (3.1)	3 (9.3)
	Second trimester (4–6 months)	116	19 (16.4)	0 (0.0)	3 (2.6)	6 (5.2)
	Third trimester (7–9 months)	152	23 (15.1)	9 (5.9)	7 (4.6)	3 (2.0)
<i>P</i> value			0.002	0.049	0.174	0.371

Table 2

The infection patterns in relation to anaemia level measured by PCV and malaria parasitaemia load in pregnant women.

Infection patterns	Examined number [<i>n</i> (%)]	Parasitaemia (parasite/μL of blood) (mean ± SD) (95% CI)	PCV (%) (mean ± SD) (95% CI)
Overall	300 (100.0)	–	39.56 ± 5.02 (31.07–36.30)
Uninfected	248 (82.7)	0.00 ± 0.0 (0.00–0.00)	39.03 ± 8.47 (36.71–40.33)
Malaria alone	14 (4.6)	1034.90 ± 234.53 (345.10–782.81)	30.24 ± 7.90 (30.05–33.67)
Malaria + <i>A. lumbricoides</i>	15 (5.0)	1005.01 ± 110.70 (537.12–751.11)	30.13 ± 9.09 (29.12–32.23)
Malaria + hookworm	11 (3.6)	859.67 ± 53.09 (375.03–994.76)	26.98 ± 4.45 (25.98–30.54)
Malaria + 2 helminths	12 (4.0)	802.04 ± 98.18 (418.01–890.55)	25.32 ± 2.76 (24.34–30.08)
<i>P</i> value	0.003	0.057	0.049

CI: Confidence interval.

Table 3

Infection pattern with respect to socio-economic and environmental factors of the pregnant women [*n* (%)].

Variables		Examined number	Malaria infection	Malaria with intestinal helminth
Overall		300 (100.0)	52 (17.3)	38 (12.7)
Educational status	Post primary school	80 (26.7)	14 (26.9)	7 (18.4)
	Primary school	78 (26.0)	17 (32.7)	13 (34.2)
	Illiterate	142 (47.3)	21 (40.4)	18 (47.4)
<i>P</i> value			0.062	0.052
Presence of stagnant water	Yes	109 (36.3)	30 (57.7)	28 (73.7)
	No	191 (63.7)	22 (42.3)	10 (26.3)
<i>P</i> value			0.070	0.041
Location of toilet facilities	Within the house	186 (62.0)	20 (38.5)	11 (28.9)
	Outside the house/bush	114 (38.0)	32 (61.5)	27 (71.1)
<i>P</i> value			0.001	< 0.001
Monthly income (Naira)	None	127 (42.3)	19 (36.5)	16 (42.1)
	5000–10000	83 (27.7)	17 (32.7)	12 (31.6)
	10500–18000	52 (17.3)	10 (19.2)	7 (18.4)
	> 18000	38 (12.7)	6 (11.5)	3 (7.9)
<i>P</i> value			0.069	0.050
Sleep outside at night	Yes	197 (65.7)	35 (67.3)	22 (57.9)
	No	103 (34.3)	17 (32.7)	16 (42.1)
<i>P</i> value			0.001	0.071
Presence of bush around habitation	Yes	185 (61.7)	21 (40.4)	25 (65.8)
	No	115 (38.3)	31 (59.6)	13 (34.2)
<i>P</i> value			0.091	0.003
Occupation	Wage earner	67 (22.3)	7 (14.5)	3 (7.9)
	Farming	103 (34.3)	18 (34.6)	15 (39.5)
	Business	71 (23.7)	11 (21.2)	6 (15.9)
	Unemployed	59 (19.7)	16 (30.8)	14 (36.8)
<i>P</i> value			0.041	0.001

4. Discussion

The current study investigated the burden of malaria and its co-infection with geohelminths among pregnant women attending two maternity hospitals in Ilorin metropolis. In sub-Saharan Africa, the incidence of co-infection of malaria and intestinal helminths is not uncommon due to the overlapping epidemiological factors that favor the transmission of the parasites[11]. In this study, 73.1% of infected individuals were co-infected with intestinal helminths. This implies that co-infection remains a serious health problem during pregnancy and may increase maternal mortality in many parts of the country[7,12]. The observed high rate of co-infection in this study may be attributable to the impairment of immunity that is associated with pregnancy. Hartgers *et al.*[13] reported that the impairment of immunity often makes primigravidae women vulnerable and susceptible to bewildering number of simultaneous infections.

The 17.3% point prevalence of malaria infection in this study is very low as compared with the output of similar studies in most parts of Nigeria[14-17]. These observations indicate that malaria-related diseases are major illness during pregnancy even when the majority of our positive cases are asymptomatic[18]. The variation in prevalence can be partially due to the behavioural activities and transmission patterns in the communities. Also, diagnostic techniques and periods of sampling may play a role in the distribution of the infections.

An important outcome in the study revealed that young primigravidae women in their first trimester recorded high incidence of malaria infection. This outcome may be ascribed to the fact that the development of immunity to infection is gradual. The higher episode of malaria infection recorded among young primigravidae women was significant when compared to old-age multigravidae women. Again, this is consistent with many studies in Nigeria and other endemic countries of the world like Kenya, Ghana and Rwanda[19-23]. Findings on co-infections of malaria with hookworm and *A. lumbricoides* are comparable particularly with ages and gestation periods of the pregnancy.

Another important finding of this study is the protective capacity in helminths infection on the intensity of malaria infection. In individuals infected with malaria, the average parasitaemia level was found to be higher when compared with pregnant women co-infected with helminths especially hookworm infections. The mean intensity of parasite further decreased in pregnant women co-infected with malaria and two helminth parasites (*A. lumbricoides* and hookworms). This is contrary to the report of Getachew *et al.*[24] who reported an increase in the clinical episode of malaria as a result of helminth infection. However, the result was in line with the findings which reported that helminths infection protects the host against severe *P. falciparum*[25]. Similarly, Nacher *et al.*[26] in a retrospective study found that people with helminths were protected against renal failure and jaundice caused by severe malaria. However, the mechanism of this synergy has not been fully established. Mwangi *et al.*[27] suggest that helminth infection modulates the inflammatory factors that protect against cerebral malaria which is a measure of parasitaemia. In addition, geohelminth infections stimulate immunoglobulin E response which activates binding CD23 and elevates NO production. Increased NO levels are found to be associated with reduced parasite sequestration and thus protect progression to severe malaria[28].

A detailed analysis of infection patterns with respect to PCV underscores malaria and its co-infection with hookworm as major contributory factors of anaemia during pregnancy in the study areas. This observation is in agreement with many studies[29-31]. According to World Health Organization's classification of anaemia, the average PCV of 30.24% recorded in patients with malaria indicates mild anaemia. However, 25.32% PCV count in pregnant women co-infected with hookworm and *A. lumbricoides* indicates severe anaemia when compared with 39.03% obtained in uninfected pregnant women[30,32]. It was further observed that as helminth infection reduces the parasitaemia level of malaria in pregnancy and, it also increases the chance of anaemic condition. This confirmed the previous observation that high prevalence of parasitic infection increases the vulnerability of pregnant and lactating mothers to anaemia[33]. Therefore, protection against malaria and intestinal parasites will contribute immensely to the prevention of anaemia and maternal mortality during pregnancy.

The role of socio-economic and environmental factors in infection pattern of malaria and soil-transmitted helminths have been well documented[34]. The rate of parasitic infections among the illiterate pregnant women was comparatively higher than the literate women. Similarly, participants who lived in homes with adequate toilet facilities have reduced prevalence of malaria and intestinal helminths. This result is in total abeyance to early studies reported in sub-Saharan Africa[35,36]. From our study, outdoor resting portends increased malaria infection as individuals who sleep outside have higher prevalence of infection as compared to those who sleep indoors. Pregnant women of low socio-economic status and inadequate housing facilities were unable to effectively prevent and control disease. This is evinced in this study where lower income earners and pregnant women that engaged in farming activities have an increased infection, which is consistent with the idea reported by Babamale and Ugbomoiko[34]. The high occurrence of parasitic infections recorded among peasant farmers, is possibly due to the nature of their occupation which exposes them to incessant bites of malaria vector and contaminated soil in their farmland.

This study underscores the burden of polyparasitism in pregnant women in high malaria transmission zone of Nigeria. Although, helminth infection has additive impact on anaemia during pregnancy, it also protects the host against severity of malaria infection. Therefore, improved environmental sanitation, provision of portable water supply and sanitary facilities coupled with administration of prophylactic drugs to pregnant women in areas of high transmission are essential preventive measures that should be advocated to ward off the debilitating consequences of malaria-helminths co-infections during pregnancy.

Conflict of interest statement

We declare that we have no conflict of interest.

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