

Full Length Research Paper

A cross-sectional study on *Plasmodium* infection: A haematological analyses of the blood samples

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A cross-sectional study on *Plasmodium* infection was conducted among 158 febrile patients (65 males and 93 females) reporting to Oyo State Hospital, Oyo, South-western Nigeria. Parasitological and haematological examinations of the blood samples were conducted. An overall infection rate of 29.7% was observed with parasite densities ranging from 96 - 64680 asexual parasites/ μ L blood. The percentages of infected male and female individuals were 27.7 and 31.2%, respectively. The least (12.5%) and the highest prevalence (44.4%) were recorded in age groups <1 and 6 - 15 years respectively. Malaria prevalence and parasitaemia were independent of age and sex ($P>0.05$). The total leucocytes and lymphocytes decreased with parasite densities, while neutrophils increased with parasitaemia but with insignificant relationships ($P>0.05$). The neutrophils and lymphocytes in infected and non-infected individuals were (54.0, 55.6%) and (45.9, 43.73%) respectively. The mean packed cell volume (PCV) of the blood in all positive cases in all age groups was lower than in negative individuals. The malaria prevalence in this study was low. Therefore, considerable efforts should further be made to reduce its occurrence below the risk level mostly among the most susceptible groups. Advocacies on the practices of Intermittent Preventive Treatment (IPT) and use of Insecticide Treated Nets (ITNs) should further be promoted.

Key words: *Plasmodium* infection, parasitaemia, prevalence, haematology, Oyo town.

INTRODUCTION

Malaria causes significant human suffering and impacts on social and economic development. There were 216 million cases of malaria, with 81% of these in the World Health Organization (WHO) African Region. An estimated 3.3 billion people were at risk of malaria in 2010 (WHO, 2011). Malaria remains a major public health problem in Nigeria where it is endemic, especially in rural populations as is the case elsewhere in Africa (Klinkenberg et al., 2005). The World Malaria Report indicated that Nigeria accounts for a quarter of all malaria cases in the 45 malaria endemic countries in Africa, showing clearly the challenge of malaria in Nigeria (WHO, 2008). This may be due to the large population; approximately 140 million

inhabitants (National Bureau of Statistics, 2006) live in areas of stable malaria transmission. Malaria results in 25% infant and 30% childhood mortality (Federal Ministry Health (FMH), 2005a). Also, 11% of maternal deaths are attributed to malaria (FMH, 2000).

More than 90% of the total Nigerian population is at risk of malaria and at least 50% of the population suffers from at least one episode of malaria each year (RBM, 2005; FHM, 2005b). The initiative 'Roll Back Malaria' launched in 1998 in partnership with the United Nations Children's Fund (UNICEF), WHO and many other non-governmental agencies seems not to be producing effective results in some malarial endemic communities of Nigeria as malaria problem is still on the increase. Many studies have reported high prevalence rates of malaria in pregnancy in different parts of Nigeria, ranging from 19.7 to 72.0% (Okwa, 2003; Adefioye et al., 2007; Kagu et al.,

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Table 1. Age and sex prevalence of malaria in febrile patients reporting to Oyo State hospital

Age (years)	Male		Female		Total	
	No. examined	No. infected (Prevalence)	No. examined	No. infected (Prevalence)	No. examined	No. infected (Prevalence)
<1	11	0 (0)	5	2 (40)	16	2 (12.5)
1-5	18	8 (44.4)	15	3 (20)	33	11 (33.3)
6-15	14	6 (42.9)	13	6 (46.2)	27	12 (44.4)
16>	22	4 (18.2)	60	18 (30)	82	22 (26.8)
Total	65	18 (27.7)	93	29 (31.2)	158	47 (29.7)

$\chi^2 = 5.72$; $P > 0.05$.

2007). With such reported high prevalence, there is a need to determine the extent of infection by *Plasmodium falciparum* in endemic communities of Nigeria as this will help in the proper management of the disease.

Haematological changes are some of the most common complications in malaria and they play a major role in malaria pathology. These changes involve the major cell lines such as red blood cells, leucocytes and thrombocytes (Maina et al., 2010). Haematological abnormalities such as anaemia, thrombocytopenia, and Leukocytosis or leucopenia have been observed in patients with malaria (Ladhani et al., 2002, Maina et al., 2010). The extent of these alterations varies with the level of malaria endemicity, background haemoglobinopathy, nutritional status, demographic factors, and malaria immunity (Price et al., 2001). Severe anaemia is the predominant severe malaria syndrome peaking in the first two years of life and is attributed to *P. falciparum* (Waitumbi et al., 2000). In malaria-infected patients, especially non-immunes children, prompt and accurate diagnosis is key to effective disease management for a favorable outcome (Miana et al., 2010).

This study reports the prevalence of malaria parasitaemia caused by *P. falciparum* and also determines the changes in the haematological parameters of the febrile patients in Oyo town in South-western Nigeria.

MATERIALS AND METHODS

Study area

The study was carried out in Oyo, an urban town in Oyo State, South-western Nigeria. It lies in the tropical rainforest belt, and according to the 1991 census had an estimated population of 3,452,720. Oyo town is made up of three Local Government Areas namely; Oyo West, Oyo East and Atiba. The native language in Oyo is Yoruba.

Study design

Ethical clearance to conduct the study was obtained from the University of Ibadan/University College Hospital Ethical Approval Committee. Prior to the commencement of the study, permission was sought from the management of Oyo State Hospital, Oyo.

Subjects included patients of all ages reporting to the hospital and directed to the hospital laboratory for blood screening for malaria parasites. All individuals that volunteered to participate were recruited for the study. Participants' consents to participate in the study were also sought and were duly informed of the significance of the study. The study was carried out between January and February, 2007, towards the end of the dry season. Parasitological and haematological examinations were done in the Oyo State Hospital, Oyo.

Preparation and examination of blood films

Blood samples were obtained from patients by trained laboratory staff on duty. Thick and thin blood films were made by spreading a drop of blood on a clean, grease-free, labelled slide and then allowed to dry. The dried blood films were then stained with 10% Giemsa stain solution and washed after 10 min using clean water. The stained films were allowed to dry and on addition of a drop of immersion oil, each slide was examined under $\times 100$ objective lens for malaria parasites. The densities of positive slides were estimated by the methods described by Cheesbrough (1999).

Haematological examination

The packed cell volume (PCV) was determined by haematocrit centrifugation technique (Jain, 1986). Total white blood cell counts and differential white blood cell counts were carried out using standard haematological techniques (Cheesbrough, 2005).

Statistical analyses

Data was entered into an Excel spreadsheet, checked for entry errors and transferred into SPSS for Windows (version 17.0, SPSS Inc, Chicago, USA) for analyses. Students' t-test was used to determine significant differences in the density of infection by *P. falciparum*. Differences in proportions were tested by Chi-square tests. Contingency Chi-square (χ^2) analysis was used to determine the association between prevalence and intensity of infection across age groups. Pearson's correlation coefficient was used to test the relationships between infection and blood parameters.

RESULTS

A total of 158 patients were screened for malaria parasites in the Oyo State Hospital laboratory. Of these, 47(29.7%) were positive to *P. falciparum*. The prevalence of infection was not significantly associated with age and sex (Table 1). The geometric mean of parasite density

Table 2. Distribution of age-related density of parasite in febrile patients reporting to Oyo State hospital.

Age (years)	Parasite density (asexual parasites/ μ L)			
	1-400	401 - 6400	>6400	Total (%)
<1	1 (2.1)	1 (2.1)	-	2 (4.3)
1-5	5 (10.6)	4 (8.5)	2 (4.3)	11 (23.4)
6-15	1 (2.1)	7 (14.9)	4 (8.5)	12 (25.5)
16>	11 (23.4)	9 (19.1)	2 (4.3)	22 (46.8)
Total	18 (38.3)	21 (44.7)	8 (17.0)	47 (100)

$\chi^2 = 7.63$, $P > 0.05$.

Table 3. Mean white blood cells (WBCs) counts, in relation to malaria infection.

Blood parameter	Leucocytes \pm SE (cell/ mm^3 of Blood)	Neutrophils \pm SE (cell/ mm^3 of Blood)	Lymphocytes \pm SE (cell/ mm^3 of Blood)
Infected	9263.96 \pm 6171.89	54.02 \pm 19.85	45.87 \pm 19.79
Non-infected	9804.26 \pm 8113.14	55.60 \pm 21.86	43.72 \pm 21.64

*The differences in the mean values of the infected and non-infected subjects were not significant ($P > 0.05$).

Table 4. Mean (\pm SE) PCV in relation to age and malaria infection status.

Age (years)	<1	1-5	6-15	16>
Infected	25.50 \pm 6.36 ^a	24.00 \pm 6.99 ^a	29.33 \pm 5.69 ^a	35.68 \pm 4.90 ^a
Non-infected	30.43 \pm 5.03 ^b	32.15 \pm 5.27 ^b	33.00 \pm 8.46 ^a	35.82 \pm 8.04 ^a

*The differences in the mean values of PCV of infected and non-infected subject was not significant ($P > 0.05$), but was significant in age groups <1 and 1 - 5 years.

was 1170 parasites/ μ L of blood. Mean parasites intensities were 7.3 ± 5.4 , 808 ± 542.4 , 689 ± 322.1 and 667 ± 364.7 asexual parasites/ μ L of blood in age groups < 1, 1-5, 6-15 and 16> years, respectively. The prevalence and intensity of infection were not age-dependent ($P > 0.05$) (Table 2).

Moreover, there were no significant variations in the mean values of the total leucocytes counts, neutrophils and lymphocytes counts in infected and non-infected individuals (Table 3). However, the mean PCV values were significantly higher in non infected individuals (32.15 ± 5.27) than in infected individuals (24.00 ± 6.99) in age group 1 - 5 years (Table 4). Total leucocytes counts and lymphocytes decreased with parasites density ($r = -0.092$, -0.07) while neutrophils increased with parasite density ($r = 0.012$), but these relationships were not significant ($P > 0.05$).

DISCUSSION

Prevalence of malaria in urban environments is generally lower than in the rural communities. The low levels of malaria incidence in the urban settlements could be as a

result of relatively good effective alert systems on malarial control. Anopheles mosquitoes may also be less abundant due to the urban pollution. However, high disease impact may result due to lack of repeated infections with multiple strains of malaria parasites in urban settings (Klinkenberg et al., 2005). Tolerance to malaria parasitaemia does occur naturally, but only in response to repeated infection with multiple strains of malaria, especially among adults in areas of moderate or intense transmission conditions (Färnert et al., 2009; WHO, 2010).

The overall prevalence of malaria in this study was low (29.7%). The value was higher than 17 and 7.7% overall prevalence reported by Anumudu et al. (2006) and Oyibo et al. (2009), among the University of Ibadan campus students and pregnant women in Lagos, South-western Nigeria, respectively. On the other hand, the overall prevalence of malaria reported in this study is substantially lower than previous estimates from other studies in peri-urban areas of Nigeria and other parts of West Africa (Ojo and Mafiana, 2005; Umeaneato and Ekejindu, 2006; Maina et al., 2010). This rather low prevalence could be attributed to the dry season during which the study was carried out. High rainfall and humidity increases mosquito

longevity and give room to the collection of clear, still, sun exposed waters, all of which enhance malaria transmission, serving as good vector breeding sites (Bremar, 2001).

Moreover, in this study, it was found that female individuals have a higher risk of being infected with malaria compared to the male participants. This is in accordance with other reports (Ibekwe et al., 2009; Okonko et al., 2009; 2010). However, the reverse trend has been reported in some other studies (Askling et al., 2005; WHS, 2006; Abdullahi et al., 2009). Attitudes of women such as getting up before dawn to perform household chores may expose them more to mosquitoes and consequently to malaria infection than their male counterparts (Vlassoff and Manderson, 1998). In addition, pregnant women are more attracted to the bites of *Anopheles gambiae* complex, the predominant African malaria-carrying mosquito, than did their non-pregnant counterparts and other population groups due to some physiological and behavioral changes that occur during pregnancy (Lindsay et al., 2000). Generally, the study showed gradual increase in prevalence with age with the highest prevalence recorded in age group 6 - 15 years. However, our study contradicted other findings that showed higher prevalence among children (<15 years of age) (Umar and Hassan, 2002; WHO, 2005). The 44.4% prevalence in the age group 6 - 15 years reported in this study corroborates with the WHO (2004) and UNAIDS/WHO (2009) 42.7% prevalence. This age group therefore constitutes the group with significantly high risk of malaria. The low malaria prevalence (26.8%) among the adults (15> years) could be due to the acquisition of immunity after continued exposure from multiple malaria infections over time (Plebanski and Hill, 2000).

The haematological abnormalities previously reported included changes in haemoglobin, leukocyte count, platelet abnormalities resulting in defective thromboplastin, and disseminated intravascular coagulation (DIC) (Richards et al., 1998). In this study, although leukocytosis was frequently seen in the malaria-infected subjects, no significant difference in WBC was found between the two groups. In contrast, other studies have demonstrated leucopenia (Erhart et al., 2004; Lathia and Joshi, 2004) or leukocytosis (Ladhani et al., 2002). These findings are comparable with those of other studies (Bashawri et al., 2002; Maina et al., 2010) that reported no significant difference in WBC between the malaria infected and non-infected groups. Meanwhile, our results on lack of association between malaria and neutrophil count deviated from previous studies that showed significantly higher neutrophil count in children with malaria compared to the non-malaria infected children (Bashawri et al., 2002; Ladhan et al., 2002; Maina et al., 2010). Higher lymphocyte count reported among malaria infected subjects in our study was also in contrast to other studies that showed that decrease lymphocyte count was associated with malarial parasites infection

(Richard et al., 1998; Erhart et al., 2004). The high level of lymphocytes could be due to its distribution into the peripheral blood during malarial infection.

Anaemia is one of the most common complications in malaria, especially in younger children and pregnant women in high transmission areas (Menendez et al., 2000). It is thought to result from a combination of haemolytic mechanisms and accelerated removal of both parasitized and non-parasitized red blood cells, depressed and ineffective erythropoiesis (Weatherall et al., 2002). Generally, the present study showed higher susceptibility of children in the age group 0 - 15 years to anaemic condition than the adult population. The significantly lower PCV values recorded among the *P. falciparum* parasitized younger children (0 - 5 years) further explain the implication of malaria in causing anaemia in the group. The low PCV values recorded among few non-parasitized subjects may in part reflect poor nutritional status, background haemoglobinopathy, intestinal worm infestation and previous and/or repeated malaria infections in this area (Maina et al., 2010).

In conclusion, malaria prevalence in the study area was low compared to some other investigations. Although being an urban setting, effective system could be responsible for the low level. However, this cannot be fully justified until similar study is carried out in the study area during the rainy season. This will enable us to evaluate the influence of seasons in the dynamics of the disease.

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