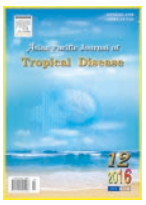
HOSTED BY

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

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



Malaria research doi: 10.1016/S2222-1808(16)61161-1

Severe malaria parasitaemia and its effects on hemoglobin and CD4$^+$ cells of HIV infected pregnant women at Kaduna State, Nigeria

Idris Abdullahi Nasir[1,2*], Olajide Olubunmi Agbede[2], Mustapha Bakare[1], Muhammad Musa Babandina[1]

[1]*Department of Medical Laboratory Services, University of Abuja Teaching Hospital, Gwagwalada, P.M.B. 228, Abuja, Nigeria*

[2]*Department of Medical Microbiology and Parasitology, College of Health Sciences, University of Ilorin, Ilorin, Nigeria*

ARTICLE INFO

Article history:
Received 12 Sep 2016
Received in revised form 21 Sep, 2nd revised form 22 Sep 2016
Accepted 15 Oct 2016
Available online 27 Oct 2016

Keywords:
Anemia
Lymphopaenia
Plamodiasis
Sydemism

ABSTRACT

Severe malaria and HIV coinfection is a disastrous syndemism especially in the face of antimalarial resistance and pregnancy. This case-control study investigated the effects of severe malaria parasitaemia (SMP)/HIV coinfections on hemoglobin concentration and CD4$^+$ cell counts of pregnant women attending four government-owned secondary hospitals in Kaduna State, Nigeria. Eighteen HIV-infected women with SMP served as test subjects while 23 HIV-uninfected women with SMP served as control subjects. All test subjects were on first-line antiretroviral therapy. Antimalarial susceptibility testing (using chloroquine, artesunate, artether and sulfadoxin-pyrimethamine), CD4$^+$ cell counts and hemoglobin concentration were conducted using schizont maturation assay, flow cytometry and methemoglobin methods respectively. Multidrug-resistant severe malaria parasitemia (MDRSMP) was defined by resistance against three or more antimalarial drugs. Eight (44.4%) women with SMP/HIV coinfections and none of the control subjects had MDRSMP respectively. There was statistical association between MDRSMP in test and control subjects ($P = 0.015$). Women with SMP had significantly low hemoglobin concentration [(7.1 ± 1.8) g/dL] and low CD4$^+$ cell counts [(209.0 ± 43.0) cells/mm^3] when compared with the control counterparts [(10.8 ± 2.2) g/dL and (431.0 ± 57.4) cells/mm^3] ($P < 0.05$). SMP/HIV coinfection was significantly correlated with hemoglobin concentration ($r = –1.25$, $P = 0.03$) but not with CD4$^+$ cell counts ($r = –2.44$, $P = 0.075$). SMP/HIV coinfections exist in our study area. In the absence of appropriate and prompt clinical interventions, these may lead to severe anemia and CD4$^+$ lymphopenia.

1. Introduction

Severe malaria and HIV coinfection is a disastrous syndemism especially in the face of antimalarial resistance and pregnancy. Malaria and HIV are two important global health infectious diseases. Malaria is the fourth leading cause of death of children less than 5 years and pregnant women in developing nations[1]. Malaria cases tend to increase each year because of poor healthcare delivery systems, emergence of drug and insecticide resistance and climate changes[2].

Geographically, Malaria and HIV/AIDS coinfections overlap, primarily in sub-Saharan Africa, Southeast Asia and South America. Pregnant women suffer particularly serious consequences when infected with both HIV/AIDS and malaria. HIV/AIDS can increase the adverse effects of malaria in pregnancy, including anemia, placental malaria infection and low birth weight[3]. HIV and malaria interact synergistically with each other. HIV infection can increase the severity of malaria and the parasite burdens might facilitate higher rates of malaria transmission. Individuals considered semi-immune to malaria in endemic regions can also develop clinical malaria if they are infected with HIV[3].

Malaria infection is associated with heightened CD4$^+$ cell activation and up-regulation of proinflammatory cytokines, providing an ideal microenvironment for the spread of HIV in CD4$^+$ cells and thus for rapid HIV replication[4]. Understanding the human immune response to malaria and HIV leads us to expect

*Corresponding author: Idris Abdullahi Nasir, Department of Medical Laboratory Services, University of Abuja Teaching Hospital, Gwagwalada, P.M.B. 228, Abuja, Nigeria.
Tel: +2348030522324
E-mail: eedris888@yahoo.com

This study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethical Research Committee of the Kaduna State Ministry of Health, Nigeria. Written informed consent was obtained from all participants.

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

that either infection might influence the clinical course of the other. The immune deficiency caused by HIV infection should, in theory, reduce the immune response to malaria parasitaemia and therefore increase the frequency and severity of clinical attacks of malaria. So HIV infection affects the clinical presentation, severity and response to treatment of malaria cases[4].

We report the findings of a case-control study by determining the effects of severe malaria/HIV coinfection on the hemoglobin and $CD4^+$ cell counts in pregnant women living in Kaduna State, Nigeria.

2. Materials and methods

The case-control study was conducted in four secondary healthcare facilities in Kaduna State, Nigeria. Blood samples anticoagulated with ethylenediaminetetraacetic acid were collected from consented pregnant women attending General Hospital Kawo, Yusuf Dantsoho Hospital, Gwamna Awon Hospital and Barau Dikko Hospital, Kaduna State, Nigeria. The selection of health facilities was based on easy accessibility and number of antenatal attendance. Eighteen HIV-infected women with severe malaria parasitaemia (SMP) served as test subjects while 23 HIV-uninfected women with severe malaria served as control subjects. All the test subjects were on first-line antiretroviral therapy (ART). Malaria microscopy and HIV screening were conducted based on standard protocols while antimalarial susceptibility tests (using chloroquine, artesunate, artether and sulfadoxin-pyrimethamine), $CD4^+$ cell counts and hemoglobin concentration were conducted using schizont maturation assay, flow cytometry and methemoglobin methods respectively. Multiple-drug resistant severe malaria parasitemia (MDRSMP) was defined by resistance against three or more antimalarial drugs.

2.1. Determination of $CD4^+$ cell count and hemoglobin concentration

$CD4^+$ cell count in whole blood was determined using Partec™ Cyflow analyzer model SL3 based on manufacturer's instruction. As described by Cheesborough[5], hemoglobin concentration was determined using methemoglobin method.

2.2. Parasite identification and counts

The parasite species were identified by Giemsa stained thin blood films and the parasite count was made against white blood cells per field on microscope slides as described by Cheesbrough[5]. Severe malaria was defined by parasite counts > 10 000/μL of blood.

2.3. Ethical approval and informed consent

This study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the ethical research committee of the Kaduna state Ministry of Health, Nigeria. Written informed consent was obtained from all participants.

2.4. Statistical analysis

Mean ± SD was derived from test and control subjects. *Chi*-square, student's *t*-test and Pearson's correction were used to determine significance between variables and study groups using SPSS software version 20 (California Inc., USA). A two sided $P < 0.05$ at 95% confidence interval was considered statistically significant.

3. Results

Eight (44.4%) women with SMP/HIV coinfections had MDRSMP. None of the control subjects had MDRSMP. There was statistical assoction between MDRSMP in test and control subjects (Table 1). Women with SMP had significantly low hemoglobin concentration [(7.1 ± 1.8) g/dL] and low $CD4^+$cell counts [(209.0 ± 43.0) cells/mm^3] when compared with the control counterparts [(10.8 ± 2.2) g/dL and (431.0 ± 57.4) cells/mm^3] ($P < 0.05$) (Table 2). SMP/HIV coinfection was significantly correlated with low hemoglobin concentration ($r = –1.25$, $P = 0.03$) but not with $CD4^+$ cell counts ($r = –2.44$, $P = 0.075$) (Table 3).

Table 2
Comparison of hemoglobin concentration and $CD4^+$ cell counts of test and control subjects.

Parameter	Test subjects ($n = 18$)	Control subjects ($n = 23$)	P
Hemoglobin (g/dL)	7.1 ± 1.8	10.8 ± 2.2	< 0.000 1*
$CD4^+$ cell count (cells/mm^3)	209.0 ± 43.0	431.0 ± 57.4	< 0.000 1*

Data are expressed as mean ± SD. *: Significant difference as determined by unpaired student's *t*-test.

Table 3
Correlation between hemoglobin concentration and $CD4^+$ cell counts in test and control subjects.

Parameter	Test subjects ($n = 18$)		Control subjects ($n = 23$)	
	r	P	r	P
Hemoglobin (g/dL)	–1.25	0.030	–0.254	0.125
$CD4^+$ cell count (cells/mm^3)	–2.44	0.075	0.016	0.913

r: Pearson's correlation.

SMP in test subjects was significantly correlated with low hemoglobin concentration but not with $CD4^+$ cell counts. However, SMP in control subjects was not significantly correlated with low

Table 1
In-vitro antimalarial resistance pattern in pregnant women infected with severe malaria [*n* (%)].

Group	No. of resistant to artemeter	No. of resistant to chloroquine	No. of resistant to artesunate	No. of resistant to sulfadoxin-pyrimethamine	No. of MDRSP (resistant to three or more antimalarial drugs)
Test ($n = 18$)	17 (94.4)	18 (100.0)	10 (55.6)	11 (61.1)	8 (44.4)
Control ($n = 23$)	8 (34.8)	20 (86.9)	6 (26.1)	20 (86.9)	0 (0.0)
P	0.000 4*	0.323 5	0.110 2	0.122 1	0.001 5*

*: Significant association as determined by *Chi*-square test.

hemoglobin concentration and $CD4^+$ cell counts.

4. Discussion

The present study has yielded some important findings in regard of malaria and HIV infections among pregnant women in Nigeria. The increased risk for severe malaria in HIV-infected persons already has been reported from areas in Nigeria[6,7].

In our study, despite the pregnant women with SMP/HIV coinfection were on first-line ART, the mean $CD4^+$ count was < 350 cells/mm^3 while the mean $CD4^+$ cell count of subjects with severe malaria without HIV infection was > 350 cells/mm^3. This is in conformity with previous reports[8,9]. The major reason for this observation may be due to the fact that patients present themselves very late for medical consultation. The part of healthcare workers with fear of stigmatization and inadequate counseling favour late consultations and inappropriate management of SMP/HIV co-infected patients. More so, the relatively low $CD4^+$ cells count in the test subjects could also be due to ART resistance, which consequently enables the HIV to be in full replication without being checkmated hence resulting in the depletion of $CD4^+$ cells.

SMP/HIV co-infected patients had anemia thus confirming several other literatures[7,10,11]. Anemia remains a major indicator profuse hemolysis and bone marrow suppression caused by malaria and HIV respectively. However, it should be noted that anemia could be multifactorial, iron deficiency and malnutrition, which could be comorbid causes[6,12].

Findings from this study showed significant correlation between hemoglobin concentration and $CD4^+$ cell count in SMP/HIV co-infected patients. This is in consistence with a report by Kakisingi *et al.*[12]. The low $CD4^+$ cells count in the test subjects could be connected with progressive immune deterioration caused by dual pathogens[13].

The rate of antimalarial resistance in subjects with SMP/HIV coinfection was relatively higher than those with SMP without HIV infection. This is contrary to reports by Byakika-Kibwika *et al.*[14], Tagoe and Boachie[13] where they reported HIV infections were not a significant risk factor for antimalarial resistance. The difference could be due to the nature of study design and the sample size.

Although the present study has yielded some important findings in regard of malaria and HIV coinfections among pregnant women, its design is not without limitations. First, as a case-control design nature does not allow us to make rigid inferences from these findings. The second limitation concerns the non-exclusion of confounded variables (*e.g.* nutrition status) that could interfere with hemoglobin value and the absence of real follow-up due to the difficulty to enroll patients.

SMP/HIV coinfection in pregnant women exists in the study area. In the absence of prompt and appropriate clinical interventions, SMP/HIV coinfection may likely lead to severe anemia and progression to AIDS. Malaria infected pregnant women especially those with HIV coinfections, which should be periodically and closely monitored for presence of antimalarial and ART resistance.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Rowe AK, Rowe SY, Snow RW, Korenromp EL, Schellenberg JR, Stein C, et al. The burden of malaria mortality among African children in the year 2000. *Int J Epidemiol* 2006; **35**(3): 691-704.

[2] World Health Organization. WHO evidence review group: intermittent preventive treatment of malaria in pregnancy (IPTp) with sulfadoxine-pyrimethamine (SP). Geneva: World Health Organization; 2012. [Online] Available from: http://www.who.int/malaria/mpac/sep2012/iptp_sp_erg_meeting_report_july2012.pdf [Accessed on 28th July, 2016]

[3] Ayisi JG, van Eijk AM, ter Kuile FO, Kolczak MS, Otieno JA, Misore AO, et al. The effect of dual infection with HIV and malaria on pregnancy outcome in Western Kenya. *AIDS* 2003; **17**(4): 585-94.

[4] Alemu A, Shiferaw Y, Addis Z, Mathewos B, Birhan W. Effect of malaria on HIV/AIDS transmission and progression. *Parasit Vect* 2013; **6**(2): 18-24.

[5] Cheesbrough M. *Medical laboratory manual for tropical countries: microbiology*. Vol 2. London: Elsevier Health Sciences; 1992, p. 234-5.

[6] Savage EJ, Msyamboza K, Gies S, D'Alessandro U, Brabin BJ. Maternal anaemia as an indicator for monitoring malaria control in pregnancy in sub-Saharan Africa. *BJOG* 2007; **114**(10): 1222-31.

[7] Onyenekwe CC, Ukibe N, Meludu SC, Ilika A, Aboh N, Ofiaeli N, et al. Prevalence of malaria as co-infection in HIV-infected individuals in a malaria endemic area of Southeastern Nigeria. *J Vector Borne Dis* 2007; **44**(4): 250-4.

[8] Ukibe NR, Onyenekwe CC, Ahaneku JI, Meludu SC, Ukibe SN, Ilika A, et al. Packed cell volume and serum iron in subjects with HIV-malaria co-infection in Nnewi, South-Eastern Nigeria. *Int J Biol Chem Sci* 2010; **4**(2): 471-8.

[9] Rutto EK, Nyagol J, Oyugi J, Ndege S, Onyango N, Obala A, et al. Effects of HIV-1 infection on malaria parasitemia in milo sub-location, Western Kenya. *BMC Res Notes* 2015; **8**: 303.

[10] Tay SC, Badu K, Mensah AA, Gbedema SY. The prevalence of malaria among HIV seropositive individuals and the impact of the co-infection on their hemoglobin levels. *Ann Clin Microbiol Antimicrob* 2015; **14**(5): 10.

[11] Sanyaolu AO, Fagbenro-Beyioku AF, Oyibo WA, Badaru OS, Onyeabor OS, Nnaemeka CI. Malaria and HIV co-infection and their effect on haemoglobin levels from three health-care institutions in Lagos, Southwest Nigeria. *Afr Health Sci* 2013; **13**(2): 295-300.

[12] Kakisingi C, Mukuku O, Manika M, Mutombo A, Kasamba E, Tshikuluila B, et al. Immunological, virological, parasitic and biological profile of malaria/HIV co-infection in 18 years old and above patients in Lubumbashi (DR Congo). *OALib J* 2016; **3**(1): e2647.

[13] Tagoe DN, Boachie J. Assessment of the impact of malaria on $CD4^+$ T cells and haemoglobin levels of HIV-malaria co-infected patients. *J Infect Dev Ctries* 2012; **6**(9): 660-3.

[14] Byakika-Kibwika P, Ddumba E, Kamya M. Effect of HIV-1 infection on malaria treatment outcome in Ugandan patients. *Afr Health Sci* 2007; **7**(2): 86-92.