Original Article

Plasmodium falciparum and intestinal parasitic co-infections in HIV-infected patients in Benin City, Edo State, Nigeria

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Abstract

Introduction: Human co-infection with *Plasmodium falciparum* and helminthes is ubiquitous throughout Africa. This study aimed to determine the co-infections of *Plasmodium falciparum* infection in HIV and intestinal parasitic infections, and their immunological distribution, in Benin City, Nigeria.

Methodology: A total of 2,000 stool specimens from HIV-positive patients and 500 controls (HIV-negative individuals) were examined for ova, cysts, or parasites using standard procedures. In addition, patients' blood samples were analyzed for CD4 counts by flow cytometry and examined for *Plasmodium falciparum* by microscopy.

Results: The prevalence of single parasitic infection among HIV patients was 18.1% in males and 16.9% among females with no significant difference (p = 0.536) while gender was a risk factor in multiple parasitic infections (male versus female: 4.2% and 1.8% OR = 2.384; 95% CI = 1.371, 4.147) (p = 0.0025). Increasing age was not associated with increased risk of both single and multiple parasitic infections (p = 0.083; p = 0.248). $CD4^+$ T cell count less than 200 cells/µl was a risk factor for acquiring single and multiple parasitic infections among HIV patients (OR = 5.565; 95% CI = 4.136, 7.486; p = 0.0001; OR = 4.283; 95% CI = 2.424, 7.566; p = 0.0001). The most common co-infection observed was between *Plasmodium falciparum* and *Ascaris lumbricoides* 43% (10) among HIV patients.

Conclusion: This study provides evidence of co-infections between *Plasmodium falciparum* and intestinal parasites. Diagnosis of parasitic infections among HIV patients is advocated as this will enhance better management of HIV-infected patients.

Key words: co-infection; *Plasmodium falciparum*; intestinal parasites; HIV; Benin City

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Introduction

Over 40 million people are living with HIV/AIDS, the majority (more than 25 million) of whom live in sub-Saharan Africa. Up to 2.4 million deaths were recorded worldwide in 2005 [1]. People in the advanced stages of HIV infection are vulnerable to secondary infections and malignancies that are generally termed as opportunistic infections as they take the advantage of the opportunity offered by a weakened immune system [2]. In HIV-positive patients, the most clinical manifestation is chronic diarrhoea and wasting due to enteric infection [3]. It has long been recognized that co-infections between HIV and other infective agents, including parasites, influence the health status of people living with HIV/AIDS. There are a number of studies on co-infections between HIV and parasitic infections in Nigeria, but with conflicting prevalence. Human co-infection with *Plasmodium falciparum* and helminthes is ubiquitous throughout Africa, although much remains unknown about its impact on public health [4]. There is little information on the co-infections of *Plasmodium* spp. and intestinal parasites among HIV patients in Edo State. Against this background, this study focuses on these co-infections in relation to immune status. Thus the main objective of this study was to determine co-infections of *Plasmodium falciparum* and intestinal parasitic infections, gender, age, and immunological distribution among HIV patients, in Benin City, Nigeria.

Methodology

Study area and population

Edo State lies roughly between 06^o 04'E and 06^o 43'E and latitude 05^o 44'N and 07^o 34'N and occupies a land area of 17,802 km^2. The study area is located in the Midwestern part of Nigeria. It has an

estimated population of 3,218,332 [5] and the state is bound with Delta State on the south, Ondo State on the west, Kogi State on the north and Kogi and Anambra States on the east. It is located within the low rain forest zone of Nigeria and has two seasons, dry and wet. The dry season lasts from mid-October to March or April while the rainy season lasts from April to September.

The study was conducted at the University of Benin Teaching Hospital, Benin City, a teaching hospital with a referral status and center for HIV/AIDS management under the President's Emergency Plan for AIDS Relief (PEPFAR). The study spanned from August 2007 to August 2009. A total of 2,500 subjects were included in this study. The study group consisted of 2,000 (668 males and 1332 females) HIV-positive patients attending HIV clinics, and 500 (209 males and 291 females) apparently healthy HIV non-infected individuals who were contacted through HIV outreach programs in their homes and offices who served as controls Patients who were on highly active antiretroviral therapy (HAART), antiparasitic agents, and those with AIDS defining conditions were excluded from this study. The age of the study subjects ranged from 21 to 70 years (mean ± standard deviation = 37.06 ± 9.19years).

Study design

This study was performed to determine co-infections of *P. falciparum* and intestinal parasites among HIV-positive patients. The random sampling method was used to select patients to participate in this study. A pre-designed structural questionnaire was utilized to collect biodata, clinical manifestations, and socio-demographic characteristics of the subjects.

Specimen collection and processing

Blood and stool specimens were collected from each participant. Venous blood specimens were collected through the use of a dry, sterile syringe and needle; the blood was withdrawn, with minimum stasis, from a suitable vein in the arm. The blood was slowly dispensed into an ethylene diamine tetra-acetic acid (EDTA) container and mixed well [6].

Plasmodium falciparum infection was diagnosed by examination of a stained thick blood film. Briefly, thick blood films were made from each blood sample and allowed to air dry. Slides were stained in 3% Giemsa stain for 30 minutes, rinsed in tap water, and allowed to air dry. The stained films were examined for malaria parasites by microscopy using an oil immersion objective lens (x100). A total of 200 fields per film were examined [1].

The blood samples were further analyzed for $CD4^+$ T lymphocyte cell estimation using flow cytometry (Partec Gmbh, Münster, Germany). Briefly, an amount of 20 µl CD4 PE antibody was placed into a Partec test tube and 20 µl of well-mixed whole EDTA blood was added; the contents were mixed gently and incubated in the dark for 15 minutes at room temperature. This mixture was agitated during incubation every 5 minutes. Next 800 µl of CD4 buffer was added to the mixture of antibody and sample and mixed gently. This was then plugged for counting.

The stool specimens were collected in clean wide-mouthed containers. The freshly voided stool specimens were processed using the formol-ether concentration method and examined microscopically for intestinal parasites as previously described [7]. Briefly, about 1 gram of faeces was emulsified in 4 ml of formol saline and agitated. The mixture was sieved. To the filtrate, 4ml of diethyl ether was added and mixed. The mixture was spun at 3,000 rpm for one minute. The faecal debris on the side of the tube was detached with the aid of a plastic pipette and the supernatant discarded. From this sediment, saline and iodine mounts were prepared and examined for the presence of parasites. Each fresh stool sample was then preserved in 10% formol saline. From this, a concentrated smear was made on a grease-free slide and stained by a modified Ziehl Neelsen stain as previously described by Akinbo *et al.* [8]. The stained smears were examined for oocysts of *Cryptosporidium* spp, *Isospora belli* and *Cyclospora cayetanensis*. Giemsa solution stain was used in detecting the spores of *Microsporidium* species by using an earlier described method [9].

Ethical considerations

The Ethical Committee of the University of Benin Teaching Hospital, Benin City, Nigeria, approved the protocol of this study. Verbal informed consent was used because of the unease and stigma associated with illiteracy in the study area.

Statistical analysis of data

The frequency data were compared using the Chi square (X^2) test while odd ratios (OR) were calculated for each potential risk factor using INSTAT software (GraphPad Software Inc, La Jolla, CA, USA).

Results

The prevalence of a single parasitic infection among HIV-positive patients was 18.1% in males and 16.9% among females with no significant difference (p = 0.536); however, gender was a risk factor in multiple parasitic infections (male versus female: 4.2% and 1.8% OR = 2.384; 95% CI = 1.371, 4.147) (p = 0.0025) (Table 1). Increasing age was not associated with increased risk of both single and multiple parasitic infections (p = 0.083; p = 0.248). The ≥ 51 years age group showed the highest prevalence in both single and multiple parasitic infections (19.5%, 4.7% respectively) with the 31 to 40 years age group showing the least prevalence (13.2%) in single parasitic infection and the 41 to 50 years age group showing the lowest prevalence (2.2%) in multiple parasitic infections (Table 2). $CD4^+$ T cell count less than 200 cells/µl was a risk factor for acquiring single and multiple parasitic infections among HIV patients (OR = 5.565; 95% CI = 4.136, 7.486; p = 0.0001; OR = 4.283; 95% CI = 2.424, 7.566; p = 0.0001) (Table 1). There were no co-infections of *Plasmodium falciparum* and intestinal parasitic infections observed from the non-HIV subjects. More females were infected with *Plasmodium falciparum* (28.0%) and *Isospora belli* (6.7%) than their male counterparts. The frequency of infection of *Ascaris lumbricoides, Cryptosporidium parvum,* Hookworm, *Strongyloides stercoralis, Taenia spp and E. histolytica*, was higher among males than females (24.8, 22.3, 15.7, 5.0, 2.48 and 2.48 respectively) (Table 2). The most common co-infection observed was between *Plasmodium falciparum* and *Ascaris lumbricoides* 43% (10) among HIV patients (Table 3).

Discussion

The brunt of the human immunodeficiency virus (HIV) pandemic has been borne disproportionately by resource-poor regions of the world, where tropical infectious diseases continue to hold greatest sway [10]. Gender did not significantly affect the prevalence of single parasitic infection among HIV patients. This is in agreement with earlier results obtained by Akinbo *et al*. [8] but disagreed with those reported by Goselle *et al.* [11]. The reason for this may have been that an equal number of sexes were recruited for the Goselle *et al*. study. However, gender significantly affected the prevalence of multiple parasitic infections among HIV-infected patients. The reasons for these observations are not clear.

Age was not a risk factor in acquiring single and multiple parasitic infections among HIV-infected patients; however, the ≥ 51 years age group had the highest (19.5%) occurrence of single parasitic infections, followed by the 41 to 50 years age group (17.2%), then the 21 to 30 years age group (16.2%), while the 31 to 40 years age group had the least (13.2%) occurrence. These findings are consistent with those of previous reports [12, 13].

CD4 + T cell counts are used as a measure of immunity and HIV disease progression [11], and counts less than 200cells/µl increase the risk of opportunistic infections. In the current investigation, HIV patients with CD4 + T cell counts less than 200cells/µl were at risk of acquiring either single or combined parasitic infections. This finding is consistent with those of previous reports [1,12,15,16,17]. HIV attacks the CD4 cells that are responsible for individual immunity, thereby leading to lowered immune status. This may explain the results observed in this study.

A total of 346 (121 cases in male and 225 in female) episodes of single parasitic infection were detected in HIV patients, with *Plasmodium falciparum* being the most common (25.2%). *Ascaris lumbricoides* was the second commonest parasite and the most frequently detected intestinal parasite in HIV patients. Previously, other authors [14,18] also reported *A. lumbricoides* as a single parasitic infection among HIV patients.

This study offered a unique opportunity to examine malaria-intestinal parasitic infections in HIV patients. The most common co-infection was between *P. falciparum* and *A. lumbricoides*. Similar findings have been reported among a population of unknown HIV status [19,20]. The other combinations with decreasing order of prevalence were *P. falciparum* / *A. lumbricoides* / hookworm, *P. falciparum* / *S. stercoralis*, *P. falciparum* / *T. trichiura* / *Cryptosporidium, P. falciparum* / *A. lumbricoides* / *T. trichiura* as well as *P. falciparum* / *T. trichiura* or *E. histolytica* or *Cryptosporidium* or *I. belli*. To our knowledge, this study provides the first data illustrating the association between *P. falciparum* and intestinal parasitic infections in HIV-positive patients in Benin City. This association may have been a result of the host-parasite relationship, particularly in HIV infection. One of the reasons for this co-infection could be due to behavioural or environmental factors leading to increased exposure to these parasitic agents [21]. For example,

Table 1. Demographic and immunological distribution of mono- and polyparasitaemia among HIV patients

Characteristics	No. tested	No. with infection	OR	95% CI	P value
Gender (Single infection)					
Male	668	121 (18.1)	1.088	0.853, 1.389	
Female	1332	225 (16.9)	0.919	0.720, 1.172	0.536
(Co-infection)					
Male	668	28 (4.2)	2.384	1.371, 4.147	
Female	1332	24 (1.8)	0.419	0.241, 0.730	0.0025
Age (Year) (Single infection)					
21 – 30	587	95 (16.2)			
31 – 40	791	104 (13.2)			
41 – 50	453	78 (17.2)			
≥ 51	169	33 (19.5)			0.083
(Co-infection)					
21 – 30	587	12 (2.0)			
31 – 40	791	22 (2.8)			
41 – 50	453	10 (2.2)			
≥ 51	169	8 (4.7)			0.248
CD4 count (cells/µl) (Single infection)					
< 200	255	98 (34.4)	5.565	4.136, 7.486	
≥ 200	1745	176 (10.1)			< 0.0001
(Co-infection)					
< 200	255	20 (7.8)	4.283	2.424, 7.566	
≥ 200	1745	34 (1.9)			< 0.0001

OR = Odd ratio; C I = Confidence Interval

Table 2. Prevalence of single parasitic infection among HIV patients

Parasite	Male (%)	Female (%)	Total (%)
Plasmodium falciparum	63 (28.0)	24 (19.8)	87 (25.2)
Ascaris lumbricoides	30 (24.8)	49 (21.8)	79 (22.8)
Hookworm	19 (15.7)	31(13.8)	50 (14.5)
Trichuris trichiura	3 (2.5)	5 (2.2)	8 (2.3)
Strongyloides stercoralis	6 (5.0)	9 (4.0)	15 (4.3)
Entamoeba histolytica	3 (2.5)	3 (1.3)	6 (1.7)
Isospora belli	6 (5.0)	15 (6.7)	21 (6.1)
Cryptosporidium spp	27 (22.3)	47 (20.9)	74 (21.4)
Giardia intestinalis	0 (0.0)	2 (0.9)	2 (0.6)
Taenia species	3 (2.5)	1 (0.4)	4 (1.2)
Total	**121**	**225**	**346**

M vs F : OR = 1.088; 95% CI=0.8530, 1.389; F vs M : OR = 0.9188, 95% CI = 0.7202, 1.172; p = 0.5361
Keys: M vs F = Male versus Female; F vs M: Female versus Male

Table 3. Parasitic combinations observed in co-infected HIV patients

Parasitic agent	Number (%)
MP, AL, CRY	1 (4.35)
MP, AL, Tt	1 (4.35)
MP, AL, HK	3 (13.04)
MP, AL	10 (43.48)
MP, HK	1 (4.35)
MP, TT	1 (4.35)
MP, SS	3 (13.04)
MP, EH	1 (4.35)
MP, CRY	1 (4.35)
MP, IB	1 (4.35)
TOTAL	23

Key: MP= Marlaria parasite; A = Ascaris Lumricoides; HK = Hookworm; EH = Entamoeba histolytica; SS = Strongyloids stercoralis; TT = Trichuris trichiura; IB = Isopora belli; GI = Giardia intestinalis CRY = Cryptosporidium species

hookworm larvae flourish in damp soil and grass, which may be found close to stagnant water that is the breeding site for mosquitoes (vector for malaria parasites) [22].

In conclusion, this study provides evidence of co-infection in the following decreasing order of prevalence*Plasmodium* /*Ascaris lumbricoides*, *Plasmodium* / *A. lumbricoides* /hookworm, *Plasmodium* / *S. stercoralis*, *Plasmodium* /*T. trichiura* / *Cryptosporidium*, *Plasmodium* / *A. lumbricoides* / *T. trichiura*, *Plasmodium* /hookworm or *T. trichiura* or *Cryptosporidium* or *E. histolytica* or *I. belli*. Diagnosis of parasitic infections among HIV patients is advocated as this will enhance better management of HIV-infected patients.

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