Original Article

Risk factors of intestinal parasitic infections among human immunodeficiency virus-infected patients on highly active antiretroviral therapy

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Abstract

Background: Highly active antiretroviral therapy (HAART) improves immunity and reduces the occurrence of enteroparasitic infections.

Aim: This study aimed to determine the prevalence and risk factors of intestinal parasitic infection among human immunodeficiency virus (HIV) patients on HAART in Kogi State, Nigeria.

Methods: Blood and stool specimens were collected from 511 subjects including 411 HIV patients on HAART and 100 apparently healthy non-HIV individuals. The blood specimens were used to determine CD4 count and haemoglobin concentration, whereas the stool specimens were processed to detect intestinal parasites using standard techniques. Socio-demographic data were obtained with the aid of a questionnaire.

Results: *Entamoeba histolytica* was the predominant parasites recovered generally and in both genders as well as being the only parasite that was associated with immunodeficiency as measured by CD4 count <200 cell/μL ($P = 0.0059$) HIV status was a significant risk factor for acquiring intestinal parasitic infection (odds ratio = 8.213 95% confidence interval = 1.971, 34.225; $P = 0.0012$). Among the other risk factors, CD4 count <200 cell/μL ($P < 0.0001$) and farming ($P = 0.0202$) were associated with intestinal parasitic infections among HIV patients on HAART.

Conclusion: An overall prevalence of 14.4% of intestinal parasitic infections was observed among HIV patients on HAART in this study. Routine diagnosis of intestinal parasites among HIV patients on HAART is advocated.

Keywords: Human immunodeficiency virus, intestinal parasitic infection, Kogi state, risk factors

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INTRODUCTION

Acquired immunodeficiency syndrome (AIDS), referred to as 'Ugiagbe' in local parlance,[1] is caused by human immunodeficiency virus (HIV). It is characterised by progressive damage to the immune system, resulting in the development of various opportunistic infections.[2,3] The HIV has emerged as a global health problem, with serious

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medico-economic and social implications. Sub-Saharan Africa remains by far the most affected region accounting for almost 70% of the global total of new HIV infections, with 24.7 million people living with HIV in 2013.[4,5] In Nigeria, 3.2 million people are living with HIV with 210,000 deaths annually.[6]

Intestinal parasitic infections are among the most common infections worldwide and about 3.5 billion persons, mostly children, are estimated to be infected.[7] In Nigeria, intestinal parasitic infection constitutes a major public health challenge.[8] Poorly planned housing, improper waste disposal, gross environmental pollution and poor environmental situations among others are driving forces for this observation.[9,10] Illiteracy, absence of clean drinking water and poverty has been shown to promote infection with intestinal parasites.[11] These have been reported as driving forces for HIV infection.[12]

One of the main features of HIV infection is immunosuppression that leads to exposing the subject to a variety of microbial and parasitic attacks.[10] Moreover, in the tropics, there is a consistent association between HIV infection and other diseases including malaria, *Mycobacterium tuberculosis* and intestinal parasitosis.[13] Intestinal parasitic infections have been observed in HIV-infected patients,[14] although the prevalence varies based on location, the age of the study population, stage of the disease and laboratory methods used.[15] People with HIV infection are vulnerable to opportunistic infections and malignancies that take advantage of the opportunity offered by a weakened immune system.[16] Antiretroviral treatment increases the length and quality of life and productivity of patients by improving survival and decreasing the incidence of opportunistic infections in people with HIV through reduction of the viral load and increasing the level of CD4 cells.[17] With the advent of highly active antiretroviral therapy (HAART) in 1996, to control HIV infection as well as improving the immune system of these patients, reduced occurrence of enteroparasitic infections has been observed.[18] There is no report on the effect of HAART on the prevalence of intestinal parasitic infection among HIV patients on HAART in Kogi state, Nigeria. Against this background, this study was conducted to determine the prevalence and risk factors associated with intestinal parasitic infections among HIV patients on HAART in Kogi state, Nigeria

METHODS

Study population

This study was conducted at the General Hospitals and Comprehensive Health Centres in Adavi, Okene, Ajaokuta and Ogori-Magongo local government areas of Kogi state. These health institutions are centres for HIV infection management in the State. The drugs used in the HAART regimen for HIV-infected patients include zidovudine, lamivudine and nevirapine. Random sampling method was employed to select participants. Patients who are not on HAART, those on anti-parasitic agents and those with AIDS-defining conditions were excluded from the study. Apparently healthy age-matched non-HIV-infected were recruited as controls. A pre-designed structured questionnaire was used to collect biodata and socio-demographic characteristics for this study during sample collection. Informed consent was obtained from participants before specimen collection. The protocol for this study was approved by the Ethics and Research Committee of the Kogi State Ministry of Health, Kogi state, Nigeria.

Sample collection and processing

Blood and stool samples were collected from each participant. A volume of 5 ml of venous blood sample was collected from each participant into ethylene diamine tetraacetic acid (EDTA) and mixed. The blood samples were analysed for $CD4^+$ T lymphocytes cell count using flow cytometry (Partec GmBh, Münster, Germany). In brief, 20 µl of CD4 PE antibody was placed into a test tube, to this 20 µl of well mixed whole EDTA blood sample of the patient was added and mixed gently. The mixture was incubated in the dark and agitated at 5 min intervals for 15 min at room temperature. Eight hundred microliters of CD4 buffer was added and mixed gently. The Partec test tube containing the mixture was inserted to the flow cytometry for counting.

Haemoglobin concentration was determined using an auto-analyser-Sysmex KX-21N (Sysmex Corporation, Kobe, Japan). Anaemia was defined as haemoglobin concentration of <13 g/dl for males and 12 g/dl for females using the WHO criteria.[19]

Freshly produced stool specimens were processed using the formol-ether concentration technique and viewed microscopically for the presence of intestinal parasites.[20] In breif, about 1 g of feces was emulsified in 4 ml formol-saline and agitated. The mixture was sieved. To the filtrate, 4 ml diethyl ether was added and agitated. The mixture was spun at 3000 rpm for 1 min. 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.

Data analysis

The data obtained was analysed using Chi-squares test and odd ratio analysis. The statistical software INSTAT® was used for the analysis (GraphPad Software Inc., La Jolla, CA, USA).

RESULTS

Five hundred and eleven subjects including 411 HIV-infected patients on HAART and 100 apparently healthy non-HIV-infected individual that served as controls were recruited for this study. The age of the participants ranged from 19 to 62 years.

HIV status was a significant risk factor for acquiring intestinal parasitic infection (odds ratio [OR] =8.213 95% confidence interval [CI] =1.917, 34.225; $P = 0.0012$). HIV patients with CD4 count <200 cells/μL ($P < 0.0001$) and were farmers ($P = 0.0202$) had significantly higher prevalence of intestinal parasitic infection. Other risk factors did not significantly affect the prevalence of intestinal parasitic infections among HIV patients on HAART [Tables 1 and 2].

A total of 59 intestinal parasites were recovered from HIV patients on HAART and *Entamoeba histolytica* was the most prevalent generally and in both genders. *Giardia intestinalis, Taenia* species and Hookworm were only recovered from females [Table 3].

None of the intestinal parasites were significantly ($P > 0.05$) associated with anaemia [Table 4], whereas only *E. histolytica* infection was significantly associated with CD4 count <200 cells/μL (OR = 3.985 95% CI = 1.563, 10.162; $P = 0.0059$) [Table 5].

DISCUSSION

HAART has also been reported to significantly reduce the morbidity and mortality of HIV infection[21] with reduction in enteroparasitic infection.[22] Lack of data on the effect of HAART on the prevalence of intestinal parasitic infection among HIV patients on HAART in Kogi state, Nigeria, necessitated this study.

A prevalence of 14.4% of intestinal parasitic infection among HIV patients on HAART was observed in this study. This is higher than the 5.3% reported in Benin City, Nigeria.[22] The difference could be due to geographical location and the setting of the research. Among children with diarrhoea, prevalence of the infection has been reported to vary with geographical locations, regions within the same country and even over time in the same location and population.[23] This study was carried out in Kogi state, North Central, Nigeria while that of Akinbo and Omoregie[22] was carried out in Benin City, South-South, Nigeria. Akinbo and Omoregie[22] study was conducted among patients receiving care in a tertiary health institution located in an urban setting, whereas the HIV patients in this study received their care from primary and secondary health institutions located in rural settings. Although HAART has been reported to improve immune status, thereby preventing the occurrence of opportunistic parasitic infections,[24] the prevalence observed in this study was high. A range of 3.9%–6.2% has been reported among non-HIV subjects in Nigeria.[15,25] However, a prevalence of 2% was observed among non-HIV subjects in this study.

The finding that HIV status was significantly associated with intestinal parasitic infections agrees with a previous

Table 1: Effect of human immunodeficiency virus status on the prevalence of intestinal parasitic infection

Characteristics	Number of tested	Number of infected (%)	OR	95% CI	*P*
HIV status					
HIV patients	411	59 (14.4)	8.213	1.971-34.225	0.0012
Non-HIV	100	2 (2.0)			
Gender					
HIV patients					
Male	51	6 (11.8)	0.772	0.314-1.901	0.7260
Female	360	53 (14.7)			
Non-HIV					
Male	8	0	2.129	0.943-48.102	0.6736
Female	92	2 (2.2)			
CD4 counts <200 cells/μL of HIV patients					
<200	38	15 (39.5)	4.876	2.367-10.046	<0.0001
≥200	373	44 (11.8)			
Clinical manifestation					
Headache	90	7 (7.8)			0.1838
Joint pain	134	25 (18.7)			
Fever	52	9 (17.3)			
Cough	37	3 (8.1)			
Diarrhoea	13	3 (23.1)			
Asymptomatic	85	12 (14.1)			

$P<0.05$. OR: Odd ratio, CI: Confidence interval

Table 2: Risk factors of intestinal parasitic infection among human immunodeficiency virus patients

Characteristics	Number of tested	Number of infected (%)	OR	95% CI	*P*
Age (years)					
19-24	49	4 (8.2)			0.2784
25-30	121	16 (13.2)			
31-36	106	20 (18.9)			
37-42	64	7 (10.9)			
43-48	36	4 (11.1)			
49-54	18	4 (22.2)			
≥55	14	4 (28.6)			
Occupation					
Civil servants	50	13 (26.0)			0.0202
Artisans	145	17 (11.7)			
Businessmen/women	171	23 (13.5)			
Teachers	13	0 (0.0)			
Farmers	7	3 (42.9)			
Students	25	3 (12.0)			
Source of water					
Municipal	40	7 (17.5)			0.6975
Borehole	280	41 (14.6)			
Well/rain water	91	11 (12.1)			
Type of toilet					
Water cistern	112	19 (17.0)			0.4038
Pit latrine	64	11 (17.2)			
Bush	235	29 (12.3)			
Source of food					
Home	222	31 (13.9)			0.6596
Vendor	87	15 (17.2)			
Canteen/eatery	102	13 (12.7)			
Eating of seafood					
Yes	314	45 (14.3)	0.992	0.519-1.897	0.9801
No	97	14 (14.4)			
Washing of fruits					
Yes	395	58 (14.7)	2.582	0.3344-19.930	0.5622
No	16	1 (6.3)			

P<0.05. OR: Odd ratio, CI: Confidence interval

Table 3: Prevalence of intestinal parasites in relation to gender

Parasites	Male (%)	Females (%)	Total (%)
Entamoeba histolytica	4 (66.67)	23 (43.40)	27 (45.76)
Strongyloides stercoralis	1 (16.67)	7 (13.21)	8 (13.56)
Ascaris lumbricoides	1 (16.67)	15 (28.30)	16 (27.12)
Giardia intestinalis	0	5 (9.43)	5 (8.47)
Taenia species	0	1 (1.89)	1 (1.69)
Hookworm	0	2 (3.77)	2 (3.39)
Total	6 (10.17)	53 (89.83)	59

study among HAART-naïve HIV patients.[26] The studies that observed parasitic infections among HIV patients on HAART did not look at association between HIV (on HAART) and intestinal parasitic infection.[22,27,28] The main findings of these studies are lower prevalence of intestinal parasitic infections among HIV patients on HAART.

The finding that gender was not significantly associated with intestinal parasitic infections among HIV patients on HAART agrees with previous reports,[22,28] whereas similar finding among non-HIV subjects had been previously noted.[26]

HAART promotes the reconstitution of the immune system of HIV-infected persons, thereby reducing the occurrence of opportunistic parasitic infections.[18,29,30] Cellular immunity is the major defense against intestinal parasitic infections.[31] Akinbo and Omoregie[22] reported that CD4 count <200 cells/μL was significantly associated with intestinal parasitic infections among HIV patients on HAART. This was also observed in this study. Akinbo and Omoregie[22] opined that, although HAART improves immunity, HIV patients on HAART with CD4 count <200 cells/mL are still prone to intestinal parasitic infections.

In this study, the prevalence of intestinal parasitic infections was highest among HIV patients with diarrhoea. This is inconsistent with previous reports.[22,28] With the exception of occupation, other studied risk factors did not significantly affect the prevalence of intestinal parasitic infections. This is not in agreement with a previous study among HAART-naïve HIV patients where all the studied risk factors significantly affected the prevalence of intestinal parasitic infections.[26] In this study, HIV patients on HAART that were farmers had significantly higher prevalence of intestinal parasitic infections compared with their counterparts in other occupation. The process

Table 4: Effect of intestinal parasites on the prevalence of anaemia

Parasite	Anaemia (%) *n*=314	Without anaemia (%) *n*=97	OR	95% CI	*P*
Entamoeba histolytica	20 (6.4)	7 (7.2)	0.875	0.358-2.136	0.9522
Strongyloides stercoralis	7 (2.2)	1 (1.0)	2.189	0.266-18.024	0.7442
Ascaris lumbricoides	11 (3.5)	5 (5.2)	0.668	0.226-1.973	0.6638
Giardia intestinalis	4 (1.3)	1 (1.0)	1.239	0.137-11.222	0.8487
Taenia species	1 (0.3)	0	0.933	0.0377-23.107	0.5779
Hookworm	1 (0.3)	1 (1.0)	0.307	0.0190-4.956	0.9627

P<0.05. OR: Odd ratio, CI: Confidence interval

Table 5: Effect of CD4 count on the prevalence of intestinal parasitic infection

Parasite	CD4 count <200 cells/μl (*n*=38)	CD4 count >200 cells/μl (*n*=373)	OR	95% CI	*P*
Entamoeba histolytica	7 (18.4)	20 (5.4)	3.985	1.563-10.162	0.0059
Strongyloides stercoralis	2 (5.3)	6 (1.6)	3.398	0.6612-17.464	0.3487
Ascaris lumbricoides	3 (7.9)	13 (3.5)	2.374	0.6451-8.734	0.3689
Giardia intestinalis	1 (2.6)	4 (1.1)	2.493	0.2714-22.905	0.9533
Taenia spp.	1 (2.6)	0	29.880	1.195-747.07	0.1589
Hookworm	1 (2.6)	1 (0.3)	10.054	0.6157-164.18	0.4407

P<0.05. OR: Odd ratio, CI: Confidence interval

of farming increases the risk of exposure to intestinal parasitic infections.

A total of 59 intestinal parasites were recovered in this study. Generally and in both genders, *E. histolytica* was the most prevalent agent of intestinal parasitic infection among HIV patients on HAART. A previous study among HIV patients on HAART in Benin City, Nigeria, reported *A. lumbricoides* as the most prevalent intestinal parasite while *S. stercoralis* was the most prevalent in a similar study from Brazil.[27] The difference could be due to geographical location. Other intestinal parasites recovered in this study included *A. lumbricoides, S. stercoralis,* hookworm, *G. intestinalis* and *Taenia* species. *A. lumbricoides, Hookworm* and *S. stercoralis* were the only intestinal parasites recovered in Akinbo and Omoregie[22] study.

HIV patients on HAART without anaemia had approximately 1–4-fold risk (OR = 2.007 95% CI = 1.112, 3.622; *P* = 0.0294) of acquiring intestinal parasitic infection (data not shown). This implies that among HIV patients on HAART, anaemia was not associated with intestinal parasitic infection (OR = 0.498). This is not in agreement with a previous report.[22] The reason for this difference is unclear. However, the cause of anaemia in HIV-positive patients is multifactorial and includes infections, neoplasm, dietary deficiencies, blood loss, medications and antibodies to antiretroviral agents.[32,33] The finding that none of the intestinal parasitic agents were significantly associated with anaemia agrees with a previous report.[22]

Only *E. histolytica* was significantly associated with CD4 count <200 cells/μL and this finding was not consistent with a previous report among HAART-naïve HIV patients where *E. histolytica* was not significantly associated with CD4 count <200 cells/μL.[25] Among HIV patients on HAART, *A. lumbricoides* has been reported to be significantly associated with <200 cells/μL[22] – a finding that was not observed in this study.

It is important to note that opportunistic coccidian parasites were not searched for in this study and they have been reported among HIV patients on HAART.[34] This may indicate that the prevalence of intestinal parasitic infection among HIV patients may be higher than reported.

CONCLUSION

An overall prevalence of 14.4% of intestinal parasitic infection among HIV patients on HAART was observed in this study. The prevalence of intestinal parasitic infection among HIV patients on HAART were higher among those with CD4 count <200 cells/μL and those who practice farming as occupation. Routine diagnosis of intestinal parasites among HIV patients on HAART is advocated.

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Conflicts of interest
There are no conflicts of interest.

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