Original Article

Pattern and Prevalence of Intestinal Helminthiasis among Human Immunodeficiency Virus-Infected Children at the University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu State

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Abstract

Background: Children infected with the human immunodeficiency virus (HIV) may be more prone to helminthic infestation because they have depleted immunity, which increases their susceptibility to infection and infestations, even with minimally pathogenic organisms such as helminths. **Aim:** The prevalence and pattern of intestinal helminthiasis among children living with HIV attending the University of Nigeria Teaching Hospital (UNTH), Ituku-Ozalla, Enugu. **Patients and Methods:** A cross-sectional study in which 70 HIV-infected children were consecutively recruited from the Pediatric HIV clinic and matched for age and sex with 70 children recruited from the children outpatient clinic (CHOP) of UNTH Ituku-Ozalla. Stool samples of study participants were collected and analyzed using the Kato–Katz method and subsequently examined under the microscope for helminths' eggs and larvae. The worm intensity was determined using the theoretical analytic sensitivity (TAS) of 24 eggs per gram (EPG) to obtain the number of eggs per gram of feces. The $CD4^+$ count, which describes the severity of immunosuppression in HIV-positive children was determined using the PARTEC Cyflow counter for the $CD4^+$ lymphocyte count, whereas HIV screening was performed using the rapid diagnostic tests for HIV (Determine, Statpack and Unigold). Data were analyzed using IBM SPSS. **Results:** The prevalence of intestinal helminthiasis among HIV-infected and non-infected children was 27.1% and 12.9%, respectively ($P = 0.038$). HIV-positive children were more likely to have intestinal helminthiasis than HIV-negative children (odds ratio [OR] =2.525, 95% confidence interval [CI]: 1.052–6.063). *Ascaris lumbricoides* was the predominant helminthic species in both HIV-infected and non-infected groups; however, there was no statistical significance between intestinal helminthic species and HIV status ($P = 0.655$) but the severity of intestinal helminthiasis was significantly associated with decreasing $CD4^+$ count ($P = 0.028$). The risk factors for intestinal helminthic infestation examined were similar in both HIV-positive and HIV-negative children ($P > 0.05$). **Conclusion:** There was a significantly higher prevalence of helminthic infestation among HIV-infected children compared to their HIV-negative counterparts. The severity of intestinal helminthiasis was significantly associated with decreasing $CD4^+$ count.

Keywords: *Children, Enugu, HIV, intestinal helminthiasis, prevalence*

Introduction

Intestinal helminthic infestations are common worldwide, especially in poor and deprived communities.[1] It remains a major public health problem

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and one of the neglected tropical diseases that affect more than 1.5 billion people all over the world, with 56% of these infestations occurring in sub-Saharan Africa, Asia, and Latin American regions.[1,2] In Nigeria, the prevalence of intestinal helminthic infestation varies between 9.1% and 95.7%, depending on the part of the country and the type of species-specific etiological agent.[3,4]

Intestinal helminthic infestations affect all age groups though children are predominantly affected.[4] Among these children, pre-school and school-aged children are at the highest risk of severe morbidity from the disease.[4-6]

Human immunodeficiency virus (HIV) infection affects all regions of the world and is also a major global public health problem.[4,5] In 2017, an estimated 36.9 million people and 2.5 million children below the age of 15 years were infected with HIV worldwide.[4-6] The sub-Saharan Africa is the most affected, with 25.8 million people living with HIV.[5-7] The disease accounts for 3.6% and 3.0% of under-five mortality in sub-Saharan Africa and Nigeria, respectively.[4] Of all the people living with HIV globally, 9% of them are in Nigeria.[7] Nigeria accounts for 30.0% of the global burden of mother-to-child transmission (MTCT) of HIV and bears 10.0% of the global pediatric HIV/AIDS burden.[4-6] Although the seroprevalence rate in Nigeria is relatively low at 3.4%,[7] there are variations within different states of the country, with Enugu state having a rate of 4.9%.[7-10]

Human immunodeficiency virus (HIV) infection, with associated depleted immunity, predisposes to an increased susceptibility to infection, even with minimally pathogenic organisms.[6] Helminthic infestation, in contrast, results in chronic activation of the immune system depressing the T-helper 1 (Th1) function.[9] Therefore, an overlapping distribution of HIV and IH becomes an important public health issue

Some epidemiological studies have reported differences in the prevalence of helminthiasis among HIV-infected patients compared to the control.[6,10,11] However, the intensity of intestinal helminths has not been related to the severity of HIV infection, thus creating a knowledge gap and as such making it imperative that this study is conducted to fill the gap in knowledge. Also, the information from this study will provide a template for further studies.

This study determined the prevalence and intensity of intestinal helminthiasis in HIV-infected children, in comparison to those who are HIV-uninfected. It also determined the association between the intensity of intestinal helminthiasis, species-specific distribution of intestinal helminthiasis, and the $CD4^+$ count of HIV-positive children.

Methods

Study area

This study was carried out between May and August 2020, at the University of Nigeria Teaching Hospital (UNTH), located in Awgu and Nkanu West Local Government Areas (LGAs) of Enugu State.

Study design

This was a hospital-based, comparative, cross-sectional study, whereby children infected and uninfected with HIV were enrolled consecutively.

Study sites

The study was conducted at the pediatric HIV clinic of UNTH, which is supported by the Catholic Caritas Federation of Nigeria. The clinic provides trained personnel for adults and children infected with HIV.

Sample size determination

The minimum sample size for this study was calculated using the standard statistical formula for sample size calculation comparing differences in proportions (equal-sized groups) in a finite population.[12]

$$n = \frac{[\ p1\ (1\text{-}p1) + p2\ (1\text{-}p2)\ ]}{(p1 - p2),} \times \text{Cp power}$$

where n = number of subjects required in each group

p1 and p2 = prevalence proportions in the two groups from an existing study (28.6% and 20.2%, respectively).[4,5]

Cp power = constant defined by the values chosen for the P value and power.

With a P value of 0.05 and power of 95%, the Cp, power = 13.0

Substituting, $n = \frac{[0.286\ (1\text{-}0.286) + 0.202\ (1\text{-}0.202)]}{0.286 - 0.202} \times 13.0$

$n = (0.365 \div 0.085) \times 13.0$

$n = 4.29 \times 13.0 = 55.77 = 56$

Having obtained the minimum sample size of 56, the calculated sample size was increased by a non-response rate of 20% to take care of late withdrawal of consent, uncompleted interviews, and failure to a submit stool sample.

Hence, $N = n \div (1 - 0.2)$

$N = 76 \div (0.8) = 70$

Therefore, 70 subjects and 70 controls were enrolled in the study, giving a sample size of 140.

Social class classification

Parental socioeconomic classification [Appendix 1] of subjects and controls was obtained using the social classification of Oyedeji.[13]

Study population

Subjects: These were HIV-positive children aged 18 months to 18 years who attended the pediatric HIV clinic at the UNTH Ituku-Ozalla, Enugu, and who had attended the clinic at least once and were coming for follow-up visits. They were confirmed to be HIV-infected through HIV antibody or DNA polymerase chain reaction (PCR) tests, and did not take anti-helminthic medication for 3 months before the study and also gave assent or whose caregivers gave consent for the study.

Controls: These were HIV-negative and apparently healthy children who attended the pediatrics outpatient clinic on a routine follow-up basis. They were age- and sex-matched children who had not had anti-helminthic medication 3 months before the study and who gave assent or whose caregivers gave consent for enrolment into the study.

Children aged less than 18 months, chronically ill children such as those with malignancies, diabetes mellitus, sickle cell anemia, and severe malnutrition, and children whose caregivers were unsure if they received anti-helminthic in the 3 months previous to the study were excluded from this study.

Ethical approval and consent

Ethics clearance from the Health Research and Ethics Committee of UNTH, Enugu, was obtained before the commencement of the study on August 25, 2019, with reference number UNTH/CSA329/Vol. 5.

Stool sample collection

Instructions were given to the parents/guardians/older participants to go home with well-labeled, wide-mouthed specimen containers to collect fresh stool using applicator sticks or a plastic spoon (depending on the stool consistency) according to the collection protocol explained to the parents/guardian. The stool samples from the study participants were received immediately or the next morning, within 4 hours from the time the stool was passed.

Stool examination

The stool samples were analyzed within 60 min of preparation using the Kato–Katz technique, followed by microscopy by two dedicated microbiologists at the University of Nigeria Teaching Hospital's microbiology laboratory. The Kato–Katz technique was used in this study because it does not require fecal concentration procedure before stool analysis for ova of the helminth.[13] The fecal smear on the slide was then examined under the microscope within 30–60 min and the number of eggs of each species of helminth counted. The number obtained was multiplied by 24, using the theoretical analytic sensitivity of 24 eggs per gram (EPG) of stool for Kato Katz, to obtain the number of EPG of feces.

$CD4^+$ cell count estimation

Blood for estimation of $CD4^+$ cell count was collected from all HIV-positive subjects in the clinic. Three milliliters of blood were collected in EDTA bottles by the researcher, with universal precautions ensured, and sent to the HIV laboratory in UNTH, Ituku-Ozalla. The $CD4^+$ lymphocyte count was performed using a PARTEC Cyflow counter (GMBH Cyview2.4, Germany). Immunological classification of cases was performed based on age-related $CD4^+$ T lymphocyte count as not significant, mild, advanced, or severe.[14]

Data management and analysis

Analysis of the results was performed using the Statistical Package for the Social Sciences (IBM-SPSS), version 19. First, data were scrutinized for incorrectly filled information and normality of distribution of data, using frequency counts, graphical displays, Shapiro–Wilk and Kolmogorov–Smirnov tests. Descriptive analysis was used to compare the sociodemographic distribution of the subjects. This was performed using frequency, percentage, median, and interquartile range. Chi-square and Fisher's exact tests were used to test for the association between categorical variables, for example, worm species, HIV status, worm intensity, and factors associated with helminthiasis infestation. The relationship between the severity of the disease (measured with CD4 count, which was skewed) and the intensity of infestation (categorized as mild, moderate, and severe) was tested using the Kruskal–Wallis non-parametric statistic. All tests of significance were two-tailed at the 5% level of significance and confidence interval estimation of 95% and results were presented in tables.

Results

The sociodemographic characteristics of the study participants are shown in Table 1. The table shows that the two groups (i.e. HIV-infected and HIV-negative children) were similar in age and gender ($P = 1.00$). Also, the distribution according to socioeconomic status showed no significant difference ($P = 0.057$).

The prevalence of helminthiasis in the HIV-positive and HIV-negative children is shown in Table 2: The

Table 1: Sociodemographic characteristics of the study participants

	HIV Status		*P*
	Positive n (%)	Negative n (%)	
Median age in months (IQR) Age group in months	120.00 (123.00)	120.00 (123.00)	1.000
18 – 59	17 (24.30)	17 (24.30)	1.000
60 – 119	17 (24.30)	17 (24.30)	
120 – 179	18 (25.70)	18 (25.70)	
180 -- 216	18 (25.70)	18 (25.70)	
Sex			
Male	38 (54.30)	38 (54.30)	1.000
Female	32 (45.70)	32 (45.70)	
Socioeconomic class			
Lower	47 (67.10)	36 (51.40)	0.057
Middle	11 (15.70)	23 (32.90)	
Upper	12 (17.10)	11 (15.70)	

*=Mann–Whitney U test. IQR=Interquartile Range

Table 2: Association between intestinal helminthiasis and HIV status

Helminthiasis	HIV Status		*P*	OR	95% CI for OR
	Positive n (%)	Negative n (%)			
Infested	19 (27.1)	9 (12.9)	0.038	2.525	1.052 – 6.063
Not infested	51 (72.9)	61 (87.1)			

Table 3: Distribution of intestinal helminthic species and intensity in the study participants

	HIV Status Positive n=19 (%)	HIV Status Negative n=9 (%)	*P*
Specie (Helminths)			
Ascaris lumbricoides	12 (63.2)	7 (77.8)	0.187*
Ancylostoma duodenale/ Necator americanus	4 (21.1)	2 (22.2)	
Trichuris trichiura	1 (5.3)	0 (0.0)	
Mixed Infection A+H	2 (10.5)	0 (0.0)	
Intensity (Helminths)			
Mild	2 (10.60)	2 (22.30)	0.128*
Moderate	10 (52.60)	3 (33.30)	
Severe	7 (36.80)	4 (44.40)	

*=2-sided Fisher's exact test. A+H = Ascaris lumbricoides+hook worm (Ancylostoma duodenale and Necator americanus); mild intensity ≤5000EPG, moderate intensity=5000–49,000, severe intensity ≥50,000 EPG

prevalence of intestinal helminthiasis in the HIV-positive and negative children was 27.1% and 12.9%, respectively. HIV-positive children were more likely to have intestinal helminthiasis than HIV-negative children (P = 0.038). The species involved in the infestation which included *Ascaris lumbricoides, Ancylostoma duodenale/Necator americanus*, and *Trichuris trichiura* were similar between

Table 4: Association between the helminthic species and the CD4$^+$count of HIV-infected children

Helminthic specie	CD4$^+$ count (cells/mm^3) Median (IQR)	*P*
Ascaris lumbriocoides	194.00 (236.00)	0.970*
Ancylostoma duodenale/ Necator americanus	328.00 (200.00)	
Trichuris trichiura	138.00 (39.00)	
Mixed infection	164.00 (63.00)	

*Kruskal–Wallis test, IQR=Interquartile range

Table 5: Association between the helminthic infestation and the CD4$^+$count among HIV-infected children

Helminthic infestation	Degree of CD4$^+$suppression		χ^2	*P*	OR 95%CI
	Not significant or Mild n (%)	Severe or Advanced n (%)			
Infestation status			12.329	0.001	
Infested	5 (11.90)	14 (50.00)			7.4 (2.2-24.4)
Not infested	37 (88.10)	14 (50.00)			
Total	42 (100.00)	28 (100.00)			

NB: OR=Odd ratio, CI=Confidence interval

the HIV-positive and HIV-negative children (P = 0.187). Despite the fact that *Ascaris lumbricoides* is the predominant helminthic species in both HIV-infected and non-infected groups, there was no significant relationship between HIV status and the type of worm produced. Table 3. The intensity of intestinal helminths (according to the World Health Organization [WHO] grading) also did not differ significantly with HIV status (P = 0.128). However, moderate intensity was more prevalent in the HIV-positive group, whereas severe intensity was more prevalent in the HIV-negative group as shown in Table 3.

Table 4 shows the association between the helminthic species and the CD4$^+$ count among HIV-infected children. This comparison revealed no significant association between CD4$^+$ count and various helminthic species (P = 0.970). Additionally, HIV-positive children with severe or advanced immunosuppression had a higher helminthic infestation (50.0%) than those with mild or no significant HIV disease (P = 0.001) as shown in Table 5.

Discussion

The main findings of this cross-sectional study are that the prevalence of intestinal helminthic infestation is significantly higher in HIV-positive children when compared with HIV-negative children, the distribution of the intestinal helminthic species was similar in the

two groups and the CD4$^+$ count was similar across the helminthic species involved in infestation; however, a significant inverse relationship was observed between the intensity of infestation and CD4$^+$ count. The prevalence (27.1%) of intestinal helminthiasis among HIV-infected children compares favorably with previous reports.[4,10,11] For example, Orji *et al*.,[4] Modjarrad *et al.,*[10] and Wagbatsoma *et al.*[5] reported the prevalence of 28.6%, 24.9%, and 21.4%, respectively, in similar populations. However, the prevalence rates found in the index study were lower than 68.5% and 52.6% reported by Obateru *et al*.[15] and Gebretsadik *et al*.,[17] respectively. The variance in prevalence rates reported in these later studies,[11,16,17] may be due to some methodological differences with the present study. For example, Ene *et al*.,[15] and Obateru.[15] studied HIV positive children who were on highly active anti-retro viral therapy (HAART naive). This is in contrast to the HAART-exposed HIV-positive children used in the present study. Highly active antiretroviral therapy has been shown to improve the immunological state and protect against intestinal helminthic infestation.[9]

Similarly, the prevalence of helminthiasis (12.7%) observed among HIV-negative children was within the range observed in Nigerian children.[3,4,15] Variations in the values of the prevalence across studies may be explained by differences in the sample size, methods of parasite concentration, and detection and the geographical location of the study setting. For example, it is known that the survival of the ova and larva of most intestinal helminths is not favorably guaranteed in a relatively dry and hot environment.[18]

The finding of a significantly higher prevalence of intestinal helminthiasis in HIV-positive children compared to HIV-negative children (27.1% vs. 12.7%) is consistent with previous reports.[4] There are plausible explanations for this finding. Firstly, HIV is known to lower the immune system, thereby predisposing affected persons to both opportunistic and non-opportunistic gastro-intestinal infestation.[19-24] Credence is given to the above reason in the present study by the finding that the intensity of infestation was inversely related to the CD4$^+$ count. Secondly, the higher proportion of children from lower socioeconomic classes in the HIV-positive children when compared with the HIV-negative children (67.1% vs. 51.4%), respectively, may partly explain the differences observed in the prevalence in the two groups.

Despite the robust evidence that the prevalence of intestinal helminthic infestation is significantly higher in HIV-positive persons when compared with HIV-negative children, some authors have found no differences.[6,25,26] For example, Harris *et al*.[27] in the adult population did not demonstrate differences in the prevalence of intestinal helminthiasis between HIV-positive and a healthy control group. There are several differences between the study of Abaver *et al*. and the present study. Firstly, the sampled population differs in demography. Although the present study involved a pediatric population, Abaver *et al.*[27] studied the adult population. Secondly, Abaver *et al.*[27] used simple microscopy for the identification of parasitic infestation. This is known to underestimate infestation. The use of the Kato–Katz technique, as employed in the index study, has been shown to facilitate the detection and quantification of helminthic ova.

Another important finding of the present study is the inverse significant relationship observed between the intensity of infestation and the CD4$^+$ count. This finding is consistent with previous reports.[14,28] The finding supports what is widely known about the immune system and diseases. For example, it is known that as the body's immune system declines, the survival and proliferation of microorganisms increase. There are several supporting data that CD4$^+$ count below 200 cells/mm^3 is significantly associated with higher intestinal helminthic infestation.[29-32]

Some important laboratory tests were not performed. Modified ZN-staining, water–ether sedimentation method, or adhesive tape/anal swab for other helminths *including Enterobius vermicularis* respectively, as well as the Baerman technique or culture for *Strongyloides stercoralis*, were not performed. Some other important intestinal helminths could have therefore been missed. It was difficult recruiting the HIV-infected under-5-year-old when compared to other age groups. This is possible because of the impact of PMTCT, which lowered the current prevalence of HIV-infected babies.

Orji *et al*.[4] and Wagbatsoma *et al*.[5] reported similarly that the pattern of helminthiasis among study participants showed that children above 12 years of age (post-primary school age) had the lowest prevalence rate of helminthiasis in both subjects and controls. The reason is that children above 12 years may better understand the need for and practice of personal and environmental hygiene compared to younger age groups.

Conclusion

This study has shown that the prevalence of intestinal helminthiasis among HIV-positive children who attended the University of Nigeria Teaching Hospital was more than two times higher than their HIV-negative counterparts. *Ascaris lumbricoides* is the predominant helminth in both subjects and controls; however, there

was no statistical significance between the intestinal helminthic species and HIV status. The severity of intestinal helminthiasis was significantly associated with decreasing $CD4^+$ count.

Recommendation

The finding in this study about the burden of helminthic infestation in HIV-positive children reinforces the integration of routine stool examination and deworming as part of comprehensive care for children living with HIV. Targeted health education drives are needed to educate HIV-positive and negative children and their caregivers on the need to dispose of human waste safely and adopt frequent hand washing with soap for themselves and their wards.

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Conflicts of interest

There are no conflicts of interest.

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APPENDIX

Appendix I: Revised surveillance case definition for hiv infection in children[12]

Immunologic category	Age-related CD4 T lymphocyte values (% of normal)			
	<11 months (%)	12–35 months (%)	36–59 months (%)	≥5 years Cells/mm^3
Not significant	>35	>30	>25	>500
Mild	30-35	25-30	20-25	350-499
Advanced	25-30	20-25	15-20	200-349
Severe	<25	<20	<15	<200 or <15%