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Comparison of Intestinal Parasitic Infection among Adults with or Without HIV/AIDS in Yaoundé and Effect of HAART and CD4 Cells Counts

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Abstract

Introduction: HIV infection and parasitic diseases constitute an important public health problem in many sub-Saharan African countries. The aim of this study was to assess the relationships between intestinal parasites, HIV/AIDS infection and Highly Active Antiretroviral Therapy (HAART).

Methods: 332 HIV infected patients and 315 controls were recruited in a cross-sectional study. Stool and blood were collected from each participant. Stool specimen was examined in search of intestinal parasites by microscopy and blood sample was screened for HIV 1 and 2 antibodies.

Results: The infection rates of intestinal parasites were higher in patients infected with HIV (19.9%; 66/332) than in those without HIV infection (11.7%, 37/315) (P=0.005). We have identified a total of eleven species of parasites in stool specimens: *Entamoeba histolytica* (6.0%), *Blastocytis hominis* (5.9%), *Entamoeba coli* (4.9%), *Trichuris trichiura* (1.2%), *Ascaris lumbricoides* (0.8%), *Giardia intestinalis* (0.8%), *Strongyloides stercoralis* (0.6%), *Cryptosporidium parvum* (0.6%), *Isospora belli* (0.5%), *Microsporidia* (0.5%) and *chistosoma mansoni* (0.1%). Infection with *Cryptosporidium parvum*, *Isospora belli*, and *Microsporidia* sp were found only among HIV positive patients. HAART was associated with a decrease of the rate of intestinal parasitic infection. *Isospora belli* and *Microsporidia* sp were diagnosed only in patients with CD4 cells counts <200 μL.

Conclusion: The frequency of intestinal parasitic infestation was the highest among HIV positive patients. The absence of HAART increases intestinal parasitism. *Cryptosporidium parvum*, *Isospora belli* and *Microsporidia* sp were confirmed to be specific to people living with HIV/AIDS.

Keywords: Intestinal parasites; HIV/AIDS; Prevalence; Yaoundé

Introduction

In several sub-Saharan African countries, HIV infection remains the major cause of morbidity and mortality among infected individuals. The reduction of immune response caused by the virus, lead to increase susceptibility to opportunistic infections. Amongst them, opportunistic intestinal parasitic infections are the most serious and the commonest ones [1,2].

Literature indicates that the prevalence of intestinal colonization due to *Microsporidia* and *Cryptosporidium* are significantly higher among HIV-infected individuals with chronic diarrhea and CD4 lympho¬cyte counts <200 cells/μL [3,4]. It is also established that the use of Highly Active Antiretroviral Therapy (HAART) in HIV infected individuals has a profound effect on immune status and greatly reduces the risk of opportunistic infections and death [5]. In Cameroon, more than 10 million people are infected with intestinal parasites and the prevalence of HIV infection was of the order of 5.3% in 2010 [6,7]. However, there is limited number of data on co-infection of HIV and intestinal parasites as well as on the impact of HAART on the prevalence of parasitic infection in HIV positive patients. We undertook this study to compare the rates of intestinal parasites among people with or without HIV infection and to determine the frequency of intestinal parasites according to HIV therapy.

Materials and methods

This study was conducted in three hospitals in Yaoundé namely the Biyem-Assi District Hospital, the Yaoundé Central Hospital and the University Teaching Hospital. Two study populations were identified in each hospital: Group 1 was made –up of HIV positive patients followed-up in the AIDS care unit and group 2 was made-up of HIV negative patients recruited in the same hospitals. All the study subjects were interviewed using a structured questionnaire to ensure that they have not taken an anti-parasitic drug within the past six months. History of antiretroviral therapy was obtained for HIV positive group. Two clinical specimens, stool and blood were collected. The stool specimen was investigated for intestinal parasites by simple wet preparation for trophozoites, wet iodine preparation and formol-ether concentration technique [8] for helminth ova and larva. A drop of concentrate stool was also stained using modified Ziehl-Neelsen staining technique for oocysts of *Isospora belli*, *Cryptosporidium parvum* and *Cyclospora* species. Blood samples were used to confirm HIV status in each group. Determine® HIV-1 and 2 commercial kits (Abbott Laboratories, Tokyo, Japan) were used for this purpose. All the manipulations were performed according to the manufacturer's instructions. Data on antiretroviral treatment and most recent CD4+ cells count were obtained from the medical files of patients.

Ethical considerations

All authors hereby declare that Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. Experiments have been examined and approved by the appropriate ethics committee. All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Participants positive for intestinal parasites, were treated free of charge.

Statistical analysis

Data obtained were verified for consistency, coded, and computerized. Statistical analysis was performed using SPSS version 16.0 and Epi Info Version 6.1 software. Different characteristics of study population were described using mean, range and percentage. The Pearson's Chi-square test was used to compare proportions and to test for association between variables in the study groups. Tests were considered statistically significant at $P \leq 0.05$.

Results the percentage of heads of households who had attended secondary school decreased from the southern (Bipindi, 40.7% and Bélabo 22.4%) to the northern regions (Meidougou 14.7% and Dompta 18.9%).

Socio-demographic data

A total of six hundred and forty seven HIV positive or negative subjects were included in this study. The mean age of participants was 33 ± 10.98 years (range: 15 to 67 years). The majority of participants were females (422/647, 65.2%). Most of them (147/647, 22.7%) were aged 26 – 30 years. More than half of participants (363/647, 56.1%) had attended secondary school and 49.6% of them were single. Concerning HIV infection, 89 (26.8%) of male and 243 (73.2%) of female were positive versus 136 (43.2%) males and 179 (56.8%) who were negative

Parasitological data

After microscopic examination of stool specimens, 15.9% (103/647) of the study subjects were positive for at least one intestinal parasite. The incidence rate of parasitic infections among HIV positive patients was 19.9% (66/332) compared to 11.7% (55/315) in HIV negative individuals (p=0.005).

Eleven different species of parasites were recovered from the stool specimens (Table 1): *Entamoeba histolytica* (7.8% in HIV positive group versus 4.1% in HIV negative), *Blastocytis hominis* (6.3% in HIV positive group versus 5.4% in HIV negative), *Trichuris trichiura* (2.1% in HIV positive group versus 0.3% in HIV negative) ($P<0.05$) and *Entamoeba coli* (5.1% in HIV positive individuals versus 4.8 % in people without HIV) were the common ones. *Cryptosporidium parvum* (1.2%), *Isospora belli* (0.9%) and *Microsporidia* (0.9%) were found only among HIV infected patients.

Type of parasite	HIV positive (N = 332) Number (%)	HIV negative (N = 315) Number (%)	Total (N = 647) Number (%)	P-value
helminths				
Trichuris trichiura	7 (2.1)	1(0.3)	8(1.2)	0.036
Ascaris lumbricoides	4 (1.2)	1 (0.3)	5(0.8)	0.052
Strongyloides stercoralis	3(0.9)	1(0.3)	4(0.6)	0.063
Schistosoma mansoni	1(0.3)	0(0.0)	1(0.1)	-
Protozoa				
Entamoeba histolytica	26(7.8)	13(4.1)	39(6.0)	0.03
Cryptosporidium parvum	4(1.2)	0 (0.0)	4(0.6)	-
Entamoeba. Coli	17(5.1)	15(4.8)	32(4.9)	0.211
Giardia intestinalis	3(0.9)	2 (0.6)	5(0.8)	0.133
Isospora belli	3(0.9)	0(0.0)	3(0.5)	-
Blastocytis horminis	21(6.3)	17(5.4)	38(5.9)	0.035
Microsporidia	3(0.9)	0(0.0)	3(0.5)	-

Table 1: Distribution of species of intestinal parasites among study group

The frequency of intestinal parasites is low among people under HAART (17.5%) compared to those without HAART (27.2%) (P=0.049). The effects of immune status on opportunistic protozoa infection among patients with HIV infection are shown in Table 2.

Type of protozoa	< 200 Number (%)	200 < CD4+ cells count < 500 Number (%)	≥ 500 Number (%)	Total Number (%)
Cryptosporidium parvum	3 (75.0)	1 (25.0)	0 (0.0)	4 (100)
Isospora belli	3 (100)	0 (0.0)	0 (0.0)	3(100)
Blastocytis horminis	4 (19.0)	6 (28.6)	11 (52.4)	21(100)
Microsporidia sp	3 (100)	0 (0.0)	0 (0.0)	3(100)

Table 2: Distribution of opportunistic protozoan according to the level of T-CD4+ cells count

Patients with severe immune depression (CD4 cells count <200 cells/µl) are more infected than those with CD4 cells count ≥ 200 cells/µL. in addition, *Isospora belli* and *Microsporidia* were found only in the severe immune depression group.

Discussion

In developing countries, intestinal parasitic infections remain a serious public health problem, especially in HIV individuals. This study determined the incidence of intestinal parasites among HIV positive and negative individuals. The study also attempted to investigate whether the distribution of infection with intestinal parasites was affected by Highly Active Antiretroviral Therapy and if infection with some protozoa is affected by immune status. Our data revealed that the overall incidence of intestinal parasites was 15.9% among the study participants. Compared to this study, a bigger rate of intestinal parasitic infection (55%) was shown among individuals in the studies of Assefa *et al.* [9].

The frequency of intestinal parasitic infections was significantly higher among HIV infected patients (19.9%) than HIV negative ones (11.7%) even with parasites that are not usually considered as opportunistic intestinal parasites such as *Entamoeba histolytica*, *Trichurus trichiura*. Similar results were reported in studies carried out in others settings [10,11]. On the other hand, *Cryptosporidium parvum*, *Isospora belli* and *Microsporidia* sp were observed only in HIV positive participants. These results agree with others epidemiological studies related to association between HIV status and intestinal parasitic infections. However, data of these studies are higher than those of the current study. Getaneh *et al.* obtained a prevalence of 25% for a *Cryptosporidium parvum* infection in HIV positive individuals [12], Assefa *et al.* reported a *Cryptosporidium parvum* and *Isospora belli* prevalence of 20.1% and 12.2% respectively among HIV positive patients [9], and Akinbo *et al.* 22.2% and 7.8% respectively [13]. In the present study, the frequency of intestinal parasitic infections was significantly lower in patients under HAART compared to those without HAART. Similar observations were reported in the studies carried out in others sites [14-16], and can be explained by the fact that antiretroviral treatment restores the immunity status in HIV positive patients and thus, decreases the incidence of opportunistic infections. Other studies [17,18] described that the severity of HIV-related immune depression is associated with increased incidence of parasitic infections. Data obtained from the current study further confirm these findings reported by these investigators. Several studies suggested that markers of advanced HIV disease such as CD4+, T cell counts or HIV RNA plasmatic levels are associated with increased risk of infection with intestinal parasites [3,19,20]. Our results also showed that infection with protozoa seems to be more frequent in patients with severe immune depression.

Two potential limitation of the current study need to be evocate: It is possible that some parasites were not detected in this study because all the specifics techniques were not used for their search. Therefore, the prevalence of intestinal parasites among the study participants may have been underestimated. In addition, CD4 cells counts were not measured at the time of the stool specimen collection; instead, we have taken the most recent CD4 cells counts that were documented in the files of patients. In fact it might have happened that the CD4 cells counts of patients may in reality be higher (for patients who started treatment) or lower (for HAART naïve patients) than the collected data by the time of stool sample collection.

Conclusion

Our findings suggest that HAART protect HIV patient from intestinal parasitic infection. The infection rate with intestinal parasites is low in the study participants, mostly in the HIV negative group. Opportunistic protozoan infection (--------) were confirmed to infect only HIV positive individuals with severe immune depression.

Competing interests

The authors declare that they have no competing interests.

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This work was carried out in collaboration between all authors. Authors, TN, KYE and RMS designed the study. Authors KYE, NGH, RMS, and KF performed the field study. Authors DNR and TN performed the statically analysis and drafted the manuscript. All authors read and approved the final manuscript.

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