Original Article

Intestinal parasitic and candida infection associated with HIV infection in Cameroon

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Abstract

Introduction: HIV causes progressive impairment of the cellular immune system leading to increased susceptibility to infectious agents. Parasitic infestations are common in HIV-infected patients and usually lead to diarrhoea. Few studies have addressed the issue of intestinal parasites among HIV-infected persons in Cameroon. This investigation was conducted in Douala, Cameroon, to assess the prevalence of gastrointestinal parasites in HIV-infected patients, taking into account their immune status and treatment course.

Methodology: Stool and blood samples were collected from 201 HIV-positive patients for the investigation of intestinal pathogens and $CD4^+$ counts.

Results: Fifty-six (27.9%) patients harbored pathogens. The most frequent pathogens were *Candida* spp. (14.9%), *Cryptosporidium* spp. (7.5%), *Entamoeba histolytica*, and *Entamoeba dispar* (3%). The presence of pathogens was significantly associated with diarrhoea, as they were found in 48.6% of diarrhoeic stools and 23.2% of non-diarrhoeic stools (OR = 3.14, p= 0.0018). Prevalence of pathogens and diarrhoea were significantly higher in patients with $CD4^+$ counts ≤ 200 cells/µL (OR = 2.17, p = 0.0349 and OR = 8.46, p = 0.000019 respectively).

Conclusions: This study highlights the need for investigating intestinal pathogens in HIV-infected patients presenting with diarrhoea, especially when their $CD4^+$ counts are low.

Key words: intestinal pathogens, CD4+ count, HIV infection

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Introduction

Human immunodeficiency virus (HIV) causes progressive impairment of the host cellular immune system leading to increased susceptibility to infectious agents and tumors [1]. The intestinal mucosa becomes a site of significant HIV replication and destruction of $CD4^+$ cells [2]. Gastrointestinal (GI) symptoms have been observed in up to 50% of North American and European patients with AIDS and up to 90% of those in developing countries. These GI symptoms are caused by enteric pathogens including bacteria, parasites, fungi, and viruses [3]. Intestinal parasites are endemic in many regions of the world where HIV is prevalent such as sub-Saharan Africa [4]. Some factors including poverty and malnutrition can promote the spread of both infections, and attempts to improve these underlying conditions may improve the situation [5]. *Cryptosporidium* species, *Cyclospora cayetanensis, Isospora belli,* and *Microsporidia* species, previously known solely in veterinary medicine, are no longer considered commensal organisms in human, since they have been identified as common opportunistic pathogens affecting HIV-infected patients [6]. Intestinal parasites remain a main cause of diarrhoea and other GI symptoms with subsequent weight loss. However their prevalence in HIV-infected patients has dramatically decreased in countries where antiretroviral treatment is widely available [7]. Few studies have addressed the issue of intestinal parasites among HIV-infected persons in Cameroon. Our study aimed at assessing the prevalence of intestinal parasites in HIV-infected patients, taking into account their immune status and treatment course.

Methodology

Study area and population

This study was conducted from January to December 2011 in Douala, a cosmopolitan city of Cameroon. Douala has the highest rate of economic activities in the Central Africa region and HIV infection prevalence is estimated at 4.6% [8].

A total of 201 HIV-positive in- and out-clinic volunteers were enrolled upon informed consent at the Laquintinie Hospital, Military Hospital, and General Hospital. The study population consisted in 134 females and 67 males (Table 1).

Questionnaire

A questionnaire was used to collect personal data from participants concerning episodes of diarrhoea, antiretroviral treatment, and use of co-trimoxazole for the prevention of opportunistic infections.

Sample collection

Stool and blood samples were collected. Each participant in the study received a stool container and clear explanation for fecal sample collection. Blood samples were collected in ethylene diamine tetra acetic acid (EDTA) tubes.

Laboratory analysis

Each fecal sample was analyzed in three ways. First, a direct wet mount in normal saline solution (0.9% NaCl solution) was examined under a light microscope (CyScope, Partec GmbH, Görlizt, Germany) for ova, cyst and parasite detection. Second, stools were concentrated using a modified formol-ether technique whereby gasoline replaces ether [9,10]. Fecal smears were then stained following the Safranin and Kinyoun method, a modified acid-fast stain technique, to detect oocysts [11]. Third, stools were cultured on Sabouraud's dextrose agar and subsequently subjected to Gram stain for identification of *Candida* species colonies [12].

Blood samples were analyzed for $CD4^+$ T-lymphocyte cell counts, using a flow cytometer (CyFlow Counter, Partec GmbH, Görlizt, Germany). Briefly, 20 µL of phycoerythrine-conjugated monoclonal antibody to human CD4 (mAb PE MEM-241, Partec GmbH, Görlizt, Germany) were gently mixed with 20 µL of whole blood into a test tube and incubated for 15 minutes at room temperature, protected from light. Next 800 µL of no-lyse buffer (Partec GmbH, Görlizt, Germany) were added to the mixture. After homogenizing its content, the tube was plugged into the CyFlow Counter for automatic counting [13].

Statistical analyses

$CD4^+$ counts were compared based on the former treatment threshold fix at $CD4^+ \leq 200$ cells/µL and the current treatment threshold fixed at ≤ 350 cells/µL [14]. All statistical analyses were conducted using XLSTAT 2012 (Addinsoft SARL, Paris, France, 2012). Chi-2 test or Fisher exact test was used to investigate the association among prevalence of intestinal parasites, $CD4^+$ counts, antiretroviral treatment, use of Co-trimoxazole, and symptoms of diarrhoea. Odds ratio was calculated to estimate the risk attributable to different factors with confidence intervals calculated using the Woolf's method. The level of significance was set at p-value = 0.05.

Ethical approvals

The study was approved by the Institutional Ethical Review Board of the Laquintinie Hospital.

Table 1. Baseline characteristics of participants

Characteristic		Number (%)
Age (years)	<20	15 (12.43)
	20-40	94 (46.77)
	>40	92 (45.77)
Mean age ± SD (years)	38.3 ± 14.2	
Gender	Male	67 (33.33)
	Female	134 (66.67)
Sex ratio (Male/female)	0.5	
Antiretroviral treatment	Yes	99 (57.23)
	No	74 (47.77)
Hospitalized	Yes	74 (36.82)
	No	127 (63.18)

Permission was obtained from the administrators of the Laquintinie Hospital, the Military Hospital and the General Hospital. Informed consent was obtained from patients prior to their participation.

Results

A total of 201 participants provided stool samples; however, only 173 gave information on their treatment regiment and 158 accepted vein puncture of blood for $CD4^+$ counts.

One hundred and eighty three (91.33%) participants were more than 20 years old and the sex ratio was 0.5. Thirty-seven (18.4%) of the 201 stool samples presented a diarrhoeic aspect. Parasitological examination revealed the presence of intestinal pathogens in 56 (27.9%) of them.

The most frequently detected pathogens were *Candida* spp. (14.9%) and *Cryptosporidium* spp. (7.5%). Intestinal pathogens were found in 48.6% of diarrhoeic and 23.2% of nondiarrhoeic stool samples (Table 2). Diarrhoea was found to be significantly associated with intestinal pathogens infections (p = 0.0018). The prevalence of diarrhoea was more than three folds higher (OR =3.14, CI = 1.5-6.58) in infected compared to uninfected participants.

Seventy-four out of 158 participants (46.84%) showed severe immunosuppression with $CD4^+ \leq 200$ cells/µL and 22 (13.9%) of them had diarrhoea. A $CD4^+$ count lower than 200 cells/µL was found to be strongly associated with diarrhoea and infections [Chi-2 = 17.84, p = 0.000019, OR = 8.46 (2.76 - 25.96) and Chi-2 = 4.45, p = 0.0349, OR =2.17 (1.05 - 4.49) respectively]. Similarly, $CD4^+$ counts lower than 350 cells/µL was associated with higher prevalence of parasites [p = 0.02144, OR = 2.67 (1.13 - 6.29); Chi-2 = 5.29] and diarrhoea (p = 0.000077; Chi-2 = 16.16)

Table 2. Prevalence of intestinal parasites

Type of infection	Pathogens		**Fecal aspect**		
			Diarrhoeic **n** (%)	Non diarrhoeic **n** (%)	Total **n** (%)
	None		19 (9.45)	126 (62.68)	145 (72.13)
Mono infections	Protozoa	*Cryptosporidium* spp.	2 (0.99)	11 (5.47)	13 (6.46)
		Cyclospora cayetanensis	0 (0)	1 (0.49)	1 (0.49)
		Entamoeba coli	1 (0.49)	2 (0.99)	3 (1.49)
		Entamoeba histolytica / Entamoeba dispar	3 (1.49)	0 (0)	3 (1.49)
		Isospora belli	1 (0.49)	0 (0)	1 (0.49)
	Helminths	*Strongyloïdes stercoralis*	2 (0.99)	0 (0)	2 (0.99)
		Trichuris trichiura	1 (0.49)	0 (0)	1 (0.49)
	Fungi	*Candida* spp.	6 (2.98)	22 (10.94)	28 (13.93)
Co-infections	Protozoa	*Entamoeba coli* + *Cryptosporidium* spp.	0 (0)	1 (0.49)	1 (0.49)
		Entamoeba histolytica / Entamoeba dispar + *Entamoeba coli*	1 (0.49)	0 (0)	1 (0.49)
	Protozoa + Fungi	*Candida* spp. + *Entamoeba histolytica / Entamoeba dispar* + *Cryptosporidium* spp.	1 (0.49)	0 (0)	1 (0.49)
		Candida spp. + *Entamoeba histolytica / Entamoeba dispar*	0 (0)	1 (0.49)	1 (0.49)
TOTAL			**37 (18.40)**	**164 (81.59)**	**201 (100)**

(Table 3). As shown in Table 4, for both thresholds, *Candida* spp. had the highest prevalence (9.5% and 12.0%) in severely immunodepressed patients ($CD4^+ \leq$ 200 cells/µL and $CD4^+ \leq 350$ cells/µL respectively), followed by *Cryptosporidium* spp. (3.2%, 3.8%). Pathogen prevalence was higher in diarrhoeic stools from severely immunodepressed patients compared to stools of patients with higher $CD4^+$ counts.

Though diarrhoea and infection with intestinal pathogens were found to be lower in participants under antiretroviral treatment (Table 3), no significant association was found between them (Chi-2 = 0.49, p = 0.4839 and Chi-2 = 0.59, p = 0.4424 respectively). No significant association was also found between the presence of pathogens or diarrhoea and chemoprophylaxis with co-trimoxazole (p = 0.497 and p = 0.608 respectively).

Discussion

This cross-sectional study has shown that the prevalence of intestinal pathogens is 27.9% among HIV-infected patients in Douala, which is less than the 33% previously reported by Sarfati *et al*., in 2006 in Yaoundé [7]. This difference might be due to the general improvement in the management of HIV during the past five years in Cameroon [15] and the selection of participants in urban hospitals with better follow-up. Also, the wide use of co-trimoxazole for chemoprophylaxis of opportunistic infections might have contributed to a lower prevalence of intestinal parasites in our study population [16].

The most frequent pathogens were *Candida* spp. (14.9%). The specific or acquired immunity against fungal growth in tissue basically consists of cell-mediated immunity regulated by T-lymphocytes that are the main target of HIV, which may explain why these fungi are frequently diagnosed as opportunistic infections in HIV-infected hosts [17]. Changes in the qualitative or quantitative composition of the bacterial flora in the gastrointestinal tract or a deficiency in specific parameters of the host's immune system evidently enhance the virulence of opportunistic *Candida* strains [18].

In this study it was observed that most of the diarrhoea cases were due to pathogens, which correlates with results found elsewhere [19, 20]. Therefore, apart from the effects of HIV enteropathy, the presence of intestinal parasites is an important contributing factor to diarrhoea, which in the longer term could result in the wasting syndrome called "slim disease" [3,21]. In agreement with observations reported in previous studies [22,23], our investigation found that intestinal parasites associated with HIV were more likely encountered as the $CD4^+$ count fell below 200 cells/µL, resulting in a higher prevalence of diarrhoea in this category of patients. As shown by Assefa *et al*. [4], patients with severe immunodeficiency are more susceptible to acquire particular parasites and are also unable to clear established infections. The increased prevalence of diarrhoea among patients with $CD4^+$ counts $\leq$ 200 cells/µL in this study may reaffirm the view that diarrhoea is an AIDS defining condition [4]. However, it should be noted that diarrhoea can occur as an adverse effect of antiretroviral treatment, especially with protease inhibitors [24]. $CD4^+$ counts were still significantly associated with diarrhoea at the $\leq$ 350 cells/µL threshold, further justifying the World Health Organisation's recommendation to initiate antiretroviral treatment at $CD4^+$ counts $\leq$350 cells/µL [14].

In this study, we focused on opportunistic parasites of the gastro-intestinal tract. Protozoan were mostly represented by *Cryptosporidium* spp. observed in 15/201 (7.5%) and *Entamoeba histolytica / Entamoeba dispar* observed in 6/201 (3%). The only helminths found were *Strongyloïdes stercoralis* 2/201 (1%) and *Trichuris trichiura* 1/201 (0.5%). Whether this low prevalence of parasites among our population is directly due to extensive antiretroviral treatment administration, secondary to a stabilization of immune response or to the use of antihelminthic drugs, cannot be established since information on the duration of ARV treatment in the participants, the stage of the disease at which the treatment was initiated, or use of antihelminthic drugs was not collected. In fact, half of the patients included in this study received HAART, which apart from indirectly restoring the $CD4^+$ counts may have a direct anti-parasitic effect [25,26]. However, no significant correlation was found between the use of HAART and the presence or absence of intestinal parasites. This result is likely multifactorial; noncompliance with medications, viral resistance to the drugs, drug–drug interactions and decreased drug bioavailability might have played a significant role [27].

Conclusion

This study investigated the prevalence of diarrhoea and intestinal pathogens among HIV-positive individuals in relation with their immune status and antiretroviral treatment. Diarrhoea was highly prevalent among HIV-infected patients with very low

Table 3. Relationship between infected patients and diarrhoea with CD4 count, antiretroviral and co-trimoxazole treatment

Factor	CD4 count (cells/µl)						On antiretroviral treatment			On co-trimoxazole		
	<200	>200	*p*-value	≤350	>350	*p*-value	Yes	No	*p*-value	Yes	No	*p*-value
Presence of parasites	25 (15.8)	16 (10.1)	0.035*	33 (20.9)	8 (5.1)	0.021*	23 (13.3)	21 (12.1)	0.442	2 (02.7)	19 (25.7)	0.497[f]
Diarrhoea	22 (13.9)	4 (02.5)	<0.001*	26 (16.5)	0 (0)	<0.001*	16 (09.2)	15 (08.7)	0.484	2 (02.7)	13 (17.7)	0.608[f]

*: Statistically significant [f]: *p*-value from Fisher's exact test

Table 4. Relationship between *Candida* and *Cryptosporidium* group with CD4 count, antiretroviral and co-trimoxazole treatment

Parasitic agents	CD4 count (cells/µl)						On antiretroviral treatment			On co-trimoxazole		
	<200	>200	*p*-value	≤350	>350	*p*-value	Yes	No	*p*-value	Yes	No	*p*-value
***Candida* spp.**	15 (09.5)	5 (03.2)	0.007*	19 (12.0)	1 (0.6)	0.003*	13 (07.5)	10 (05.8)	0.920	0 (0)	10 (13.5)	0.341[f]
***Cryptosporidium* spp.**	5 (03.2)	6 (03.8)	0.550[f]	6 (03.8)	5 (3.2)	0.188[f]	6 (03.5)	7 (04.0)	0.403	2 (02.7)	5 (6.8)	0.584[f]

*: Statistically significant [f]: *p*-value from Fisher's exact test

$CD4^+$ cell counts and associated with GI pathogens. *Candida spp*., *Cryptosporidium* spp. and *Entamoeba histolytica / Entamoeba dispar* were the most frequent parasites. This study highlights the importance of initiating ARV treatment when $CD4^+$ cells counts remain around 350 cells/µL and underscores the need to look for intestinal parasites in HIV-infected patients presenting with diarrhoea and/or very low $CD4^+$ cells counts. Knowledge of infections of the GI tract in HIV-positive patients in Cameroon could help improve the management of these patients in the context of increased avaibility of antiretroviral treatment.

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