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THE PREVALENCE OF INTESTINAL COCCIDIAN PARASITES BURDEN IN HIV/AIDS PATIENTS ON ANTIRETROVIRAL THERAPY IN HIV CENTERS IN MUBI, NIGERIA

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ABSTRACT

Background: Intestinal coccidia are group of protozoa which parasitize the epithelial cells of the intestinal tract of their hosts. Most infections usually produce mild, self-limiting infections in man, but they now constitute a serious public health problem, especially in developing countries with inadequate sanitary conditions coupled with widespread HIV/AIDS infection.

Objective: To determine the Prevalence of intestinal coccidian parasites burden in HIV/AIDS patients on antiretroviral therapy in HIV Centers in Mubi, Nigeria

Materials and Methods: This was a hospital-based cross-sectional study in which stool specimens from HIV-positive patients on ART were examined for the presence of oocysts of intestinal coccidian parasites using Modified Acid Fast Stain technique. In addition, patients' blood samples were analyzed for CD4 count by flow cytometry and packed cell volume (PCV) through microhaematocrit centrifugation.

Results A total of 305 specimens examined, 236(77.4%) were positive for *Cryptosporidium parvum*, *Isospora belli* and *Microsporidium* species. Patients within the age group of 21 - 30 were the most infected. Generally, the duration of ART influenced the prevalence of the intestinal coccidian parasites. There was a highly significant association between the CD4 count and prevalence coccidian parasites ($p < 0.05$). There was a significant negative correlation ($r = -0.95$) between the duration of the ART and the prevalence of coccidian presence.

Conclusion: Routine screening of HIV-positive patients for intestinal parasites is advocated as standard operative procedure (SOP) before antiretroviral therapy (ART) is given. Construction of public health facilities, toilets and boreholes as well as public enlightenment campaign is recommended for more effective management of these patients.

Keywords: intestinal coccidian parasites, antiretroviral therapy, Mubi

LA PREVALENCE DES COCCIDIES INTESTINALES CHEZ LES PATIENTS ATTEINTS DE VIH/SIDA SOUS TRAITEMENT ANTIRETROVIRAL DANS LES CENTRES DE CONTROLE DE VIH DE MUBI AU NIGERIA.

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Résumé

Contexte: Les coccidies intestinales sont un groupe de protozoaires qui parasitent les cellules épithéliales du tube digestif de leurs hôtes. La plupart des infections humaines sont d'habitude peu sévères et autolimitées, mais elles constituent de nos jours un véritable problème de santé publique, particulièrement dans des pays en voie de développement où les conditions sanitaires sont inadéquates, et couplées à l'infection répandue du VIH/SIDA.

Objectif: Déterminer la fréquence de coccidies intestinales chez les patients atteints de VIH/SIDA sous traitement antirétroviral dans les Centres de contrôle de VIH de Mubi au Nigeria.

Matériel et Méthodes: Il s'agissait d'une étude transversale dans laquelle les spécimens de selles des patients séropositifs au VIH et sous traitement antirétroviral étaient examinés en vue d'en dépister la présence d'ocystes de coccidies intestinales grâce à la technique de coloration acido-résistante modifiée. De plus, les prélèvements de sang des patients étaient analysés pour en déterminer le taux de CD4 et le taux de micro-hématocrite par les techniques de flux cytométrique et de centrifugation respectivement.

Résultats: Un total de 305 spécimens ont été examinés, 236 (77.4 %) étaient positifs pour le *Cryptosporidium parvum*, le *Isoospora belli* et les espèces de Microsporidie. Les patients dans la tranche d'âge de 21 - 30 ans étaient les plus infectés. Généralement, la durée du traitement antirétroviral influençait la fréquence des coccidies intestinales. Il y avait une association fortement significative entre le taux de CD4 et les infections ($p < 0.05$). Il existait une corrélation négative significative ($r = -0.95$) entre la durée de traitement antirétroviral et la fréquence de coccidies intestinales.

Conclusion: Le dépistage de routine des patients séropositifs pour des parasites intestinaux est préconisé dans la procédure opératoire standard avant toute administration de thérapie antirétrovirale. La construction d'installations de santé publique, des toilettes et des puits de forage ainsi que des campagnes d'éducation sanitaire sont fortement recommandées en vue d'une prise en charge effective des patients atteints de VIH.

Mots-clés : coccidies intestinales, thérapie antirétrovirale, Mubi

INTRODUCTION

Gastrointestinal tract (GIT) infections constitute a serious public health problem in developing countries with inadequate sanitary conditions coupled with widespread HIV/AIDS infection. Many different types of intestinal parasites infect man, provoking a wide range of symptoms that are generally associated with gastrointestinal tract disorders and are dependent on demographic, socio-economic, physiological and immunological factors [1]. With the progressive development of AIDS, especially once CD4⁺T lymphocyte counts have fallen below 200 cells/ μ l, patients often become co-infected by bacteria, parasites or viruses [2]. Gastrointestinal tract parasitic infections often present as diarrhea and significant disease has been recorded in 50-96% of cases with 90% prevalence rate reported in Africa [3]. Infective causes of chronic diarrhea may satisfactorily be managed because with the exception of cryptosporidiosis and HIV-related enteropathy, good response to treatment can be expected [4], but all etiologic agents are not easily diagnosed in Africa on routine basis because of limited diagnostic facilities and trained personnel [5].

Following HIV infection, the virus replicates rapidly in lytic cycles inside resident tissue macrophages and CD4⁺ T lymphocytes. This results in depletion of these vital immune cells, rendering the individual susceptible to opportunistic pathogens, a host of which include intestinal protozoa, resulting in the frequently observed diarrhea in this group of persons [6]. The incidence and prevalence of infection with a particular enteric parasite in HIV/AIDS patients is likely to depend upon the endemicity of that particular parasite in the community [7]. Several parasites have been implicated as major contributors to morbidity in HIV-

infected persons living in developing countries, and the parasites frequently encountered include mostly intestinal coccidia such as: *Cryptosporidium* spp., *Isoospora belli* and *Microsporidia* spp [8]. Most of these infections in man are zoonotic (having the potential to infect animals or arise from animals). In immunocompetent individuals, they usually produce mild, self-limiting infections [9].

Cryptosporidium parvum is the species responsible for human infections and is now widely recognized as a cause of acute gastro-enteritis, particularly in children and other immunosuppressed persons such as AIDS patients [10]. The infection produces a persistent, watery, offensive diarrheic stool, often accompanied by abdominal pain, nausea, vomiting and anorexia. In immunocompetent persons, symptoms are usually short lived (1 to 2 weeks). The small intestine is the site most commonly affected. In immunocompromised individuals, especially in HIV patients, Cryptosporidiosis can be life threatening. As many as 10% of AIDS patients pass oocysts of *C. parvum* in their stools [11]. *Isoospora belli* has a cosmopolitan distribution; occurring especially in warm regions of the world infecting both humans and animals [12]. It is endemic in Africa, Asia and South America. Infection with *I. belli* manifest as diarrhea with abdominal cramp which can last for weeks and result in fat malabsorption and weight loss. In immunocompromised individuals, infants and children, infection ranges from self-limiting enteritis to severe diarrhea illness resembling that of cryptosporidiosis [13]. *Cyclospora cayentanensis* has also been described in association with diarrhea illness but the infection usually results in a disease with non-specific symptoms [14]. *Microsporidia* species are known as opportunistic pathogens in humans with Acquired Immune Deficiency Syndrome (AIDS) and have

been implicated in conditions ranging from enteritis to keratoconjunctivitis [15]. The most common *Microsporidia* found in patients with AIDS are *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis* and *Encephalitozoon hellem*. Patients with these infections tend out to be severely immuno-deficient with a CD4⁺ T cell count less than $100 \times 10^6/L$ [16]. In addition, cases of microsporidiosis have been reported in immunocompromised persons not infected with HIV and in immunocompetent individuals. The clinical manifestations of microsporidiosis are very diverse, varying according to the causal species with diarrhea being the most common [16].

Since the prevalence of parasites associated with gastroenteritis is dependent of their geographical distribution and socio-economic factors surrounding a given community, laboratory investigations are required to determine prevalence in each population in order to provide an effective planning and management policy. Human immunodeficiency virus (HIV) and parasites have long been of public health concern and have drawn global attention. However, although much work have been done on the prevalence of parasites in HIV patients, at the moment there is scarcity of information on the prevalence of intestinal coccidia among HIV positive patients in Mubi HIV centers of Adamawa State, Nigeria. The availability of such data will be an enormous contribution to the implementation of regulatory policies on the management of HIV/AIDS patients, hence this study was conducted.

MATERIALS AND METHODS

Study area

Adamawa state is located in the North Eastern part of Nigeria. It lies between latitude 7° and 11° north of the equator and longitude 11° and 14° east of the Greenwich Meridian. It is divided into 21 Local government areas [17]. The growth of Mubi town is traced to the agricultural, administrative, and commercial functions it performs. The hospitals in which this study was carried out are the three HIV specialized centers of the Mubi General Hospital which geo-politically covers Mubi North, Mubi South, and Michika Local Governments. The Hospitals care for the people living with HIV and also receives patients from other parts of the country due to their consistent

supply of reagents for CD4⁺ counts for HIV infected patients.

Sample collection

The study was cross-sectional hospital based study carried out from June to October 2013. Male and female HIV/AIDS patients visiting the hospital for treatment or routine follow-up were provided with information on the purpose/objectives of the study and its possible benefits. They were however explained that it was not an obligation for them to participate in the research and neither was it a pre-requisite to accessing routine medical or other social services publicly available. The effective study participants (ESP) were those who consented and provided stool samples for laboratory investigation and their blood samples were taken for CD4⁺ T cell determination.

Freshly voided stool samples were collected into clean wide mouth specimen containers from volunteer participants. A portion of the stool was preserved in 10% formalin in a proportion of 10g of stool in 3 mL of formalin. Five mL of venous blood were collected into coated EDTA tubes for CD4⁺ T cell counts and packed cell volume (PCV). The ESP were grouped based on their levels of immune suppression thus: Based on the CD4⁺ T cell counts, the participants were categorized by their immune status according to the 1993 Revised Classification System for the HIV Infection by CD4 T-cell categories [18]. Samples were numbered progressively for identification and these laboratory numbers ensured individual confidentiality. Participants were not billed for the tests and the physicians were provided with the findings for necessary action. The samples collected for the study as resolved with the participants were solely used for the defined purpose. The ethical clearance was approved by the Ethical Committee of the Hospital prior to sample collection.

Modified Ziehl Neelson Stain

Detection of intestinal coccidian parasites oocysts in the concentrated stool was done using the modified cold Ziehl Neelsen staining technique. A concentrated smear of the stool was made on a clean grease-free slide and fixed in methanol for 3 minutes. The slide was immersed in cold Carbol fuchsin and stained for 15 minutes. It was then thoroughly rinsed in tap water and decolorized in 1% HCl (v/v) in methanol for 10-15

minutes. After rinsing again in tap water, the slide was counterstained with 0.4% malachite green for 30 seconds. The slide was then air-dried and observed under the compound light microscope using 40x objective lens for the presence of *Cryptosporidium* oocysts, which was confirmed under the oil-immersion objectives as small pink to red spherules on pale green background.

Determination of CD4+ T cell count

Blood samples collected from the same patients who provided stool samples were analyzed for CD4+ T lymphocyte cell estimation using flow cytometry. Briefly, 20 µl of CD4 PE antibody was placed into a Partec test tube and 20µl of well-mixed whole EDTA blood was added, mixed gently and incubated in the dark for 15 minutes at room temperature. The mixture was agitated during incubation every 5 minutes. 800 µl of CD4 buffer was added to the mixture of antibody and sample and mixed gently. This was then plugged to the counter for counting.

Determination of the Packed Cell Volume (PCV)

The PCV was estimated by centrifuging a sample of well-mixed anticoagulated blood in capillary tubes using the microhaematocrit centrifuge. The machine was set at 12,000g for 5 minutes which automatically attains the correct speed. The PCV was subsequently estimated by measuring the height of the red cell column and expressing it as a ratio of the height of the total blood column. A PCV reader determined this ratio [19].

Data analysis

Data got from the processing of samples were analyzed using SPSS 12.0. The Chi-Square test was used to compare proportions at significant level of 0.05.

RESULTS

A total of 305 specimens examined, 236(77.4%) were positive for *Cryptosporidium parvum*, *Isospora belli* and *Microsporidium* species. Table 1 shows the prevalence of coccidian parasites in study participants according to gender. There was no significant difference in the prevalence by gender of participants ($P > 0.05$).

TABLE 1: PREVALENCE OF COCCIDIAN PARASITES IN STUDY PARTICIPANTS ACCORDING TO GENDER

Parasites	Number (%) of participant infected		TOTAL n=305
	Males (n=68)	Females(n =237)	
<i>Cryptosporidium parvum</i>	32 (47.0)	95 (40.1)	127(41.6)
<i>Isospora belli</i>	23 (33.8)	72 (30.4)	95(31.1)
<i>Microsporidium</i> species	2 (2.9)	10 (4.2)	12(3.9)
Total	57 (83.8)	177 (74.6)	234(76.7)

The Prevalence of coccidian parasites in study participants according to age is shown in Table 2. Patients within the age group of 21 - 50 were the most

frequently infected, though there was no significant difference in the prevalence by age ($P > 0.05$).

TABLE 2: PREVALENCE OF COCCIDIAN PARASITES IN STUDY PARTICIPANTS ACCORDING TO AGE

Parasites	Number (%) of infected participants aged (years)			Total n=305
	<21 (n=30)	21–50 (n=239)	>51 (n=36)	
<i>Cryptosporidium parvum</i>	10 (33.3)	102(42.7)	15(41.7)	127(41.6)
<i>Isospora belli</i>	9 (30.0)	74 (30.9)	12(33.3)	95(31.1)
<i>Microsporidium species</i>	1 (3.3)	11 (4.6)	0	12(3.9)
Total	20 (66.6)	187 (78.2)	27(75.0)	234(76.7)

Table 3 shows the prevalence of intestinal coccidian parasite infection according to the duration of ART by patients. There was a highly significant association

between the prevalence of intestinal coccidian parasites and to the duration of treatment by patients ($P < 0.05$)

TABLE 3: PREVALENCE OF INTESTINAL COCCIDIAN PARASITE INFECTION ACCORDING TO THE DURATION OF ART

Parasites	Number (%) of patients having been treated for							Totaln = 305
	1 yr n = 85	2 yrs n = 80	3 yrs n = 60	4 yrs n = 30	5 yrs n = 30	6 yrs n = 5	7 yrs n = 15	
<i>Cryptosporidium parvum</i>	40(47.1)	19(23.8)	31(51.7)	17(56.7)	14(46.7)	1(20.0)	5(33.3)	127(41.6)
<i>Isospora belli</i>	27(31.8)	21(26.3)	21(35.0)	8(26.7)	10(33.3)	3(60.0)	5(33.3)	95(31.1)
<i>Microsporidium species</i>	7(8.2)	1(1.3)	2(3.3)	0(0)	1(3.3)	0(0)	1(6.7)	12(3.9)
Total	74(87.0)	41(51.1)	54(90.0)	25(83.3)	25(83.3)	4(80.0)	11(73.3)	234(76.7)

Table 4 shows the prevalence of parasites in study participants according to CD4 count. There was a strong negative correlation ($r = -0.95$) between the duration of the ART and the prevalence of coccidian presence and the association between CD4 count of patients and the presence of coccidian parasites showed a high level of significance ($P < 0.05$)

DISCUSSION

In this study, there was a high prevalence (76.7%) of coccidian parasite infection in the study population conducted during . The fact that the study was conducted during the wet period could account for this unexpected high prevalence; Previous studies in Rwanda [20] and Kenya [21] have shown that peaks of infection tend to occur in the wet months. Reports from West Africa [22,23] and Zambia [24] have shown human infections peak early in the season, perhaps because susceptible populations develop immunity after repeated exposure with the initial rains. This high

prevalence can further be explained by the widespread of common open toilets, the incidence of random defecation, and the occurrence of stormy winds. It has been shown by Siobhan et al [25] that the storm water runoff during rainy seasons undoubtedly increases environmental transport of feces and wet, humid conditions favor parasite survival. Also the intensive rearing of cattle in the region and the use of ovine manure as fertilizer surely goes a long way to enhance the epidemiology of parasites. Moreover, the lack of hygiene can equally enhance the transmission of these parasites. Risk factors for acquisition of parasitic infections are the same in both immunocompetent (IC) and immunosuppressed (IS) individuals. The immune system plays an integral part in modifying the establishment of infection, controlling disease once it is established, limiting the severity and dissemination of the disease, and assisting in clearance or control of the parasite. Thus, immunosuppressed hosts are more likely to acquire infection after exposure, have more severe disease once the infection is established, have disseminated infection rather than localized infection, and be unable to clear parasites with chronic carriage states. These all lead to, and account for, the greater morbidity and mortality in these patients.

In our study, there was no significant difference in the prevalence by gender of participants. This finding disagrees with the report from Kenya [26]. The authors argued that that exposure to *Cryptosporidium* was influenced by gender, age and role in the household. Adult women had more daily contact with cattle faeces than adult men, and older women had more contact than older men. Women took more care of sick people and were more at risk from exposure by this route. We found no significant difference in the prevalence of infection with coccidian intestinal parasite by age. This finding is not in accordance with a report from India [27] in which the highest prevalence of these organisms was in the group aged 16-45 years and during the rainy months. A similar study carried out in Israel [28] reported an Age-related infection with *Cryptosporidium* species. Since these infections may be asymptomatic or present as a mild self-limited disease, many infected peoples do not seek medical aid. Consequently, under-appreciation and over-appreciation of infection with intestinal coccidians may occur depending on the diagnostic technique used. Our study population comprised HIV-positive patients already on ART: it was not the case for the earlier-mentioned reports. That is probably the reason for the above disagreements.

TABLE 4: PREVALENCE OF PARASITES IN STUDY PARTICIPANTS ACCORDING TO CD4 COUNT

Parasites	Number (%) of infected patients with CD4 count			Total n= 305
	<200 n=30	200 - 499 n=201	≥ 500 n= 74	
<i>Cryptosporidium parvum</i>	14(46.7)	92(45.8)	21(28.4)	127(41.6)
<i>Isospora belli</i>	11(36.7)	65(32.3)	19(25.7)	95(31.1)
<i>Microsporidium species</i>	0(0)	8(4.0)	4(5.4)	12(3.9)
Total	25(83.3)	165(82.1)	44(59.4)	234(76.7)

Since the patients involved in this study were all on ART, one could have expected a lower parasites load, since the treatment is supposed to have boosted their immune status. Thus, comparison in this study between the parasite load and the immune status of patients based on their CD4⁺ T cell counts according to

the 1993 Revised Classification System for the HIV infection by CD4⁺ T-cell categories by Castro et al.[18] showed that 83.3% of the patients infected with the opportunistic coccidians were significantly associated with CD4 count below 200. This is in concordance with the view that the outcome of infection by enteric

protozoan parasites is dependent on absolute CD4⁺T cell counts, with lower counts being associated with more severe disease [29].

The present study showed no significant difference in the intestinal profiles of study participants using wet preparation based on their CD4⁺T cell counts. On the other hand, unlike the non-opportunistic parasites, low CD4 count (83.3% with <200 CD4 count) was highly significantly associated with the presence of coccidians, where there is a decrease in CD4 counts at the beginning of treatment and a higher prevalence of the parasites.

The mean PCV of the patients with CD4 count less than 200 was significantly lower compared to those with CD4 count within 200 and 500, and those with CD4 above 500. Though one cannot ascribe the patients in this study to be anemic, previous studies have shown that CD4⁺T cell count of less than 200cells/ μ L were associated with an increased risk of parasitic infection among HIV-infected patients, and co infection with Malaria, a risk to anemia [30]. This is important as CD4 count of less than 200cells/ μ L is believed to be associated with disease progression and opportunistic infections. This may explain the association between CD4 count and parasitic infections among HIV patients with anemia.

CONCLUSION

The prevalence of Coccidian parasites in HIV/AIDS patients attending Mubi HIV centres is relatively high. Female patients were more infected with commonly identified parasites through wet preparation while there was no significant difference in infection rates with opportunistic parasites. The age range most affected in this study was between 21 to 40 years, with the peak between 21 to 30 years for the opportunistic coccidians. As the duration of treatment

increased, the parasitic load decreased. There was no significant difference in the intestinal profiles of study participants using wet preparation while the prevalence of patients infected with the opportunistic coccidians was highly significantly associated with CD4 count. There was no significant difference between CD4 count and PCV, though lower PCV was equally associated with low CD4 counts. Public health measures should continue to emphasize the importance of environmental and personal hygiene as well as provide and monitor the source and quality of drinking water. This is a very important point as the community depends a lot on wells as sources of water and the prevalent wind storms disseminate the pathogens throughout the localities. Stool processing should be a routine in HIV/AIDS patients attending treatment centers and more training should be given to health practitioners and laboratory technicians about opportunistic emerging parasites.

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AUTHORS'

CONTRIBUTIONS

Armand Claude Noundo Djieyep conceived and designed the study, conducted the literature search, drafted the manuscript and carried out the laboratory investigations. Felicité Djieyep Djemna assisted in the design and the laboratory investigations. Delphine Leila Davids supervised the research work. Benjamin Thumamo Pokamp participated in the literature search. Henri Lucien Kamga substantially revised the manuscript and prepared it for publication. All authors read and approved the manuscript.

COMPETING INTERESTS

The authors declare having no competing interests.

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