Original Article

DIARRHOEA, CD4 CELL COUNTS AND ENTERIC INFECTIONS AMONG HIV PATIENTS ATTENDING A HOSPITAL IN JOS, NIGERIA

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ABSTRACT

Enteric pathogens' associations between diarrhoea and CD4 cell counts were studied. Stool specimens were obtained from HIV-infected patients with diarrhoea. Out of the 600 stool samples analysed 339(56.50%) were positive for parasites. Protozoa accounted for 227(37.83%) while helminthes were 112(18.67%) of infection identified. The opportunistic parasite *Cryptosporidium* was the most frequently identified Protozoon parasite 128(24.67%) followed by *Giardia lamblia* 38(6.33%). As at enrollment, CD4 counts among patients were significantly ($p<0.005$) lower in those with diarrhoea than in those without diarrhoea. There was a significant association ($p<0.05$) between diarrhoea and low CD4 counts. Over two-thirds of diarrhoeal episodes were undiagnosed, suggesting that unidentified agents or primary HIV enteropathy are important causes of diarrhoea in this population. There is a strong negative association between duration of diarrhoea and CD4 levels.

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INTRODUCTION

The prevalence of intestinal pathogens among Human Immune-deficiency virus (HIV)–infected individuals has dramatically decreased in countries where antiretroviral agents are widely available (le Moing *et al.,* 1998; Carr *et al.* 1998). However, in most African Countries, few patients have access to antiretroviral therapy. Intestinal pathogens still represent a frequent cause

of diarrhoea, weight loss, and muscular wasting. Prevalence rates of intestinal parasites, including opportunistic protozoans, in Africa vary from study to study depending on the diagnostic technique used and study population. The information on the cause of diarrhoea and the possibility of isolation of parasites has largely come from various cross-sectional studies (Born, *et al.,* 2006; Enriquez and Sterling, 1993; Mengeshe, 1994). Expectedly, infectious etiologies lead the list in developing nations in contrast to non-infectious etiologies in developed nations. In most of this studies, the emphasis was on chronic diarrhoea, acute episodes and risk factors being largely unaddressed. Presently such information is available from two large prospective community based cohort studies that reported on rates of diarrhoea and isolation of enteric pathogens in developing nations (Mwachari *et al.*, 1998). There are many reports regarding frequency of various pathogens causing diarrhoea from different parts of Nigeria, Some studies also demonstrated regional variability of Pathogens (Khumalo-Ngwenya *et al.*, 1994), as well as changing trend of etiology in the same population (from infectious to non infectious). However, reports regarding correlation of diarrhoea with CD4 counts and impact of CD4 levels on isolation of pathogens have not been studied in Nigeria. The aim of this study was to find a relationship between diarrhoea and CD4 counts, types of diarrhoea and variation in frequency of enteric parasites in HIV patients.

MATERIALS AND METHODS

HIV/AIDS patients attending the Faith Alive Foundation were involved in this study. Six hundred (600) stool samples were obtained from HIV patients (aged from 1years to 60years) presenting with symptoms of diarrhoea. An informed consent was taken from all the patients, which included consent to do HIV testing, CD4 estimation, and recording of the clinical details, as well as performing invasive and non-invasive tests which were already an existing practice in the hospital.

Definition of Diarrhoea

Symptoms reported by patients were used to classify diarrhoea into acute and chronic episodes. Diarrhoea was defined as the passage of two or more unformed stools in the past 24hours. An episode of diarrhoea was classified as acute if it lasted for less than a month and provided the patient is diarrhoea free in the proceeding month and chronic when diarrhea lasted more than a month, or was intermittent and recurrent over a period of at least two months with diarrhoeal symptoms for at least half this time. Subsequent diarrhoea was classified as a new episode if there was diarrhoea–free interval of at least one month. If the duration of symptoms did not fit any of these definitions the case was excluded from the analysis.

Sample Analysis

Samples were collected from the hospital's Microbiology/Parasitology laboratory. Information on the duration of symptoms of diarrhoea was obtained from the case records cards maintained at the clinic. For patients with chronic diarrhoea, fresh specimens were examined directly using macroscopic technique. A small portion of the stool was emulsified in a drop of normal saline on a microscope slide and another portion in a drop of Lugol's iodine on another slide. These wet smears were examined under the x100 and x400 magnification for intestinal parasites. The presences of *Isospora* and *Cryptosporidium* oocysts were confirmed by examination of the stool specimen by modified Zielh-Neelsen's stain. The smears were fixed with methanol for 1 minute, flooded with carbol fuchsin for 15 minutes and decolorizing was done by 1% acid alcohol for five minutes. Counter staining was done with 0.4% malachite green for 1 minute. Microscopic examination was carried out under low power magnification, (x100) and high power (x100) lenses. Intense red, pink or faint pink, round or oval oocysts were observed.

HIV Serology

HIV status of the patients was confirmed by ELISA method using two different antigens.

CD4 count

CD4 counts were measured routinely during the first and the follow- up visits.

For this study, each time a participant provided a stool sample their most recent CD4 count was used for the analysis.

Data Analysis

Analysis was performed using SPSS version 13.0 statistical software. The relationship between the CD4 counts of the patients and duration of diarrhoea symptoms were assessed using t-test. A univariate analysis was performed to look at the association between parasites isolated and diarrhoea. An adjusted analysis, using a logistic regression model, was then performed to address the association between parasites isolated and patients' CD4 counts (dichotomized in two groups :< 200 cells/µl)

RESULTS

Of the 600 stool samples of HIV/AIDS patients analysed, 339(56.50%) parasites were identified (Table 1). Protozoa accounted for 227(37.83%) including *Entamoeba histolytica* 37(6.20%), *E. coli* 3(0.50%), *Giardia lamblia* 26(4.30%), *Cryptosporidum sp* 148(40.33%), *and Isospora belli* 13(2.20%), while helminthes were 112(18.67%) of infection identified including *Ascaris lumbricoides* 32(5.30%), *Dyphylibothriun latum* 9(1.50%), *Taenia spp* 11(1.80%), *Strongyloides stecoralis* 7(1.20%), *Trichuris trichuria* 6(1.00%), *Schistosoma mansoni* 17(2.80%), Hookworms *30(5.0%)(*Table 1).

CD4 Counts and Types of Diarrhoea

Out of the 600 samples analysed 214(35.67%) patients had diarrhoea. Patients with acute or chronic diarrhoea have significantly (p<0.005) lower CD4 cell counts than those without diarrhoea, and patients with chronic diarrhoea had lower CD4 counts than those with acute (Table 2). There was a strong negative association between the duration of diarrhoea and CD4 levels. It was also observed that most patients with low CD4 had cryptosporidiosis (Fig. 1).

Table 1: Prevalence of intestinal parasites in HIV/AIDS patients attending a Hospital in Jos.

Parasites	HIV-positive (600)
Protozoa	No. +ve (%)
Entamoeba histolytical	37(6.20)
Entamoeba coli	3 (0.50)
Giardia lamblia	26(4.30)
Cryptosporidium sp.	148(49.30)
Isospora belli	13(2.20)
Helminthes	
Ascaris lumbricoides	32(5.30)
Diphylobothrum latum	9(1.50)
Teania spp.	11(1.80)
Strongyloides stercuralis	7(1.20)
Trichuris tricuria	6(1.00)
Schistosoma mansoni	17(2.80)
Hookworms	30(5.00)

Table 2: CD4 Cell counts in relation to types of Diarrhoea in HIV/AIDS patients attending a Hospital in Jos

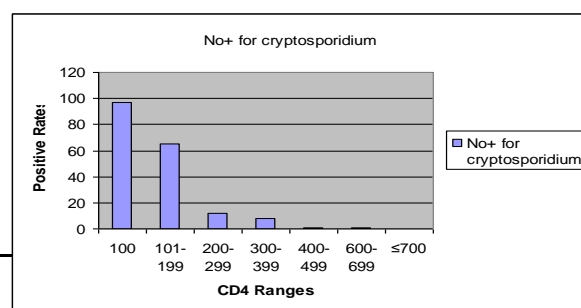| Type of diarrhea	No. positive	CD4 Ranges
Acute	97	200-299
Chronic	117	100-199
No. diarrhea	86	>300

Figure 1:CD4 ranges in relation to the prevalence of *Cryptosporidium* sp.

DISCUSSION

Diarrhoea is well recognized as an important component of HIV related morbidity and thus, included in the clinical case definition of AIDS (NACO and WHO, 1991)). In this hospital based prospective study of patients living in and around Jos - Nigeria, an attempt was made to establish the etiological risk factors, and rates of diarrhoea. The result of the study have confirmed that diarrhoea is common among HIV patients, and it has a strong association with CD4 counts, particularly, chronic diarrhea. Similarly a few factors

including CD4 levels, stool characters, and duration of diarrhoea affected the isolation rates of parasites from stool. Majority of the study population were at their advanced stage of HIV infection (AIDS), confirmed by clinical stages and CD4 cell level. The rates of 35% diarrhoea episodes was substantially greater than the diarrhoea episodes observed in South-South Nigeria cohort study supporting the view that HIV-associated diarrhoea disease occurs more commonly also in Zambia (Navin *et. al.,* 1999; Weber *et al.,*1999). Rates of identification of organism from stool sample were less when compared to other studies (Brink *et al., 2002)*.The pathogens were more frequently associated with chronic rather than acute diarrhea.

It was observed that the CD4 cell counts influenced the cause of diarrhoea. The frequency of infections is low in patients with higher CD4 counts. These may be due to the effective HAART which helps in eradicating opportunistic protozoal infection, and associated with the influx of CD4 positive cells into the lamina propria.

It was observed in the present study that both of chronic and acute diarrhoeal episodes were experienced by patients with CD4 counts of less than 200cells/µl, indicating that it is a frequent problem even in less advanced stages of HIV. The results of the study had substantially higher positivity rates than that of a similar study in India (Jadhavv, 2005).

CONCLUSION

The population in Jos North, North Central Nigeria, had higher diarrhoea episodes, protozoa etiologies dominated over others in this population, watery stool is known to have a better diagnostic yield, and Strong relation was observed between acute diarrhoea, short duration of symptoms and negative diarrhoea with CD4 levels.

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