Research Article

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Seasonal variation of microsporidiosis among HIV-infected persons in Benin City, Nigeria.

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The prevalence of microsporidiosis in relation to seasonal variations is unknown. This study is aimed at determining the correlation between seasonal variation and the prevalence of microsporidiosis in HIV-infected persons in Benin City, Nigeria. A total of 763 persons consisting of 463 HIV-infected patients attending HIV clinics and 300 apparently healthy HIV non-infected persons were recruited in this study. Stool specimens were collected from each patient and processed using standard procedure. The prevalence of microsporidiosis was significantly associated with season. More episodes of intestinal parasitic infections were observed in the rainy season than the dry season (OR = 1.990; 95% CI = 1.374, 2.882; p = 0.0004). This study underscores the association between microsporidiosis and seasonal variations with higher prevalence during the rainy season.

Key words: Benin City, HIV, microsporidiosis, Seasonal variation.

Microsporidia species is one of the common causes of chronic diarrhea in AIDS patients (van Gool *et al*., 1995; Tumwine *et al*., 2002) which is responsible for unexplained weight loss in HIV- infected persons (Sokolova *et al*., 2011). The clinical manifestations of microsporidiosis are diverse and include intestinal, pulmonary, ocular, muscular, and renal disease (Didier and Weiss, 2006). The correlation between seasonal variation and the prevalence of microsporidiosis is still under investigation. A study in the United States (Conteas *et al*., 1998) and another in Brazil (Cotte *et al*., 1999) did not observe seasonal trend in the prevalence of microsporidiosis. Data are lacking on the association between seasonal variation and the prevalence of microsporidiosis in HIV-infected persons in Nigeria. This study is aimed at determining the correlation between seasonal variation and the prevalence of microsporidiosis in HIV-infected persons in Benin City, Nigeria.

MATERIALS AND METHODS

Study population

The study was carried out at the University of Benin Teaching Hospital, Benin City – a teaching hospital with a referral status and center for HIV/AIDS management under the United States President's Emergency Plan for AIDS Relief (PEPFAR). A total of 763 persons consisting of 463 HIV-infected patients attending HIV clinics and 300 apparently healthy HIV non-infected individuals that were contacted through HIV outreach programs in their homes and offices that served as controls were included in this study.

Individuals that were on highly active antiretroviral therapy (HAART), antiparasitic agents and those with AIDS defining conditions were excluded from this study. Verbal informed consent was obtained from each participant. This study was approved by the Ethical Committee of the University of Benin Teaching Hospital, Benin City.

Specimen collection and processing

Stool specimens were collected from each participant into clean wide-mouthed container. The freshly voided stool specimens were processed using formol-ether concentration method and examined microscopically for intestinal parasites as previously described (Cheesbrough, 1999). Briefly, about 1g of faeces was emulsified in 4ml of formol saline and agitated. The mixture was sieved. To the filtrate, 4ml of diethyl ether was added and mixed. The filtrate-ether mixture was spun at 3,000rpm for 1 minute. The faecal debris on the side of the tube was detached with the aid of a plastic pipette and the supernatant discarded. From this sediment, saline and iodine mounts were prepared and examined for presence of parasites. Following this, each fresh stool sample was preserved in 10% formol saline. From this, a concentrated smear was made on glass slide and stained by Ryan's modified Trichrome stain as previously described (Patil *et al*., 2008).

Briefly, smear was fixed in absolute methanol for 10 minutes and allowed to air-dry. The air dried smear was stained in modified trichrome stain for 90 minutes and differentiated in 1% acid-alcohol for 3 seconds. Smear was rinsed in two grades of alcohol (95%, 100%), 5 minutes in each. The stained smears were examined microscopically for the spores of microsporidia. The rainy season was defined as the period between the months of April and September while the dry season was between October and March.

Statistical analysis

The data obtained were analyzed using Chi square (X^2) to compare the frequency of data. The odd ratio was calculated for potential risk factor. The software INSTAT (GraphPad Software Inc., La Jolla, CA, USA) was used in all statistical analysis.

RESULTS AND DISCUSSION

Generally, the prevalence of microsporidiosis was significantly associated with season. More episodes of intestinal parasitic infections were observed in the rainy season than the dry season (OR = 1.990; 95% CI = 1.374, 2.882; p = 0.0004). Out of the intestinal parasites observed in this study, only *Microsporidia* was associated with rainy season (Table 1). The least episode of infection was observed in *Entamoeba histolytica* in both rainy and dry seasons (Table 1).

Microsporidia are one of the leading causes of diarrhea in people living with HIV (Weber *et al*., 1994; Schwartz *et al*., 1996). With the emergence of AIDS microsporidial diarrhea has gained significance, as it is one of the important causes of morbidity and mortality (Tuli *et al*., 2008). It has been suggested that seasonal variations in the occurrence of intestinal protozoan infections holds epidemiological significance (Tuli *et al*., 2008). Hence, this study aims at determining seasonal variations in the occurrence of microsporidiosis.

Of the eleven different types of parasites recovered, only microsporidia species was significantly associated with seasonal variation with higher prevalence in the rainy season.

Bodies of water and animals are known sources of microsporidial infection (Weiss, 2001; Slodkowicz-Kowalska *et al*., 2006; Cama *et al*., 2007). It is possible that droppings from wild and domestic animals which may contain microsporidial spores can easily be washed by surface-running water after rainfall into streams and rivers. These bodies of water are used as sources for drinking and domestic use (Akinbo *et al*., 2010). This will increase the chances of microsporidial infections among immunodeficient persons. This may explain the finding in this study.

The finding of association between microsporidial infection and seasonal variation is of epidemiological importance. This information is important in understanding the transmission, control management of microsporidiosis among HIV-infected patients. It is important to note that only *Ascaris lumbricoides* and hookworm were recovered from non-HIV infected patients and neither was associated with seasonal variations.

Table 1: Seasonal trend on the prevalence of intestinal parasitic infections in HIV-infected persons.

Organism	Rainy (%)	Dry (%)	OR	95% CI	P value
E. histolytica	2 (0.8)	3 (1.4)	0.590	0.098, 3.565	0.896
Isospora belli	11 (4.5)	5 (2.3)	2.003	0.685, 5.859	0.300
Microsporidia	61 (24.5)	28 (12.8)	2.250	1.376, 3.677	0.002
A. lumbricoides	39 (15.9)	28 (12.8)	1.285	0.761, 2.170	0.420
Hookworm	18 (7.4)	11 (5.0)	1.492	0.688, 3.234	0.408
S. stercoralis	9 (3.7)	5 (2.3)	1.625	0.536, 4.925	0.553
T. trichiuris	4 (1.6)	7 (3.2)	0.500	0.144, 1.733	0.419
Taenia spp	0	4 (1.8)	0.097	0.005, 1.815	0.104

In conclusion, this study underscores the association between microsporidiosis and seasonal variations with higher prevalence during the rainy season. A clear understanding of this seasonal dynamics may be helpful in preventing infection.

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