

A Pilot Study Of Microsporidiosis And CD4 T-Cells In North- Central, Nigeria

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Abstract: Two species of Microsporidia were identified (*Enterocytozoon bieneusi* and *Encephalitozoon cuniculi*) among the non-HIV, and immunocompromised individuals at the Jos, Plateau and Benue State, Nigeria. In Jos, 500 species were subjected to laboratory test and the infection rate of microsporidium species were 1(0.5%) for gastroenteritis with <350 cell/μl; 15(15.0%) were HIV individuals with <200 cell/ μl, and 19(19.0%) AIDS patients had low CD4 counts (<100 cell/ μl). The severity of the illness was seen to have impact on the CD4 counts of the individuals in Makurdi where, in both sexes 5(3.5%) students had <100 cells/ μl CD4 counts with corresponding fall in 36(5.7%) Civil servants of <100 cells/ μl cells count. Housewives were 44(8.8%) with <50 cells/ μl, and others (undefined occupational status) 11(1.5%) had CD4 counts <90 cell/ μl. Microsporidiosis is now known to cause a serious threat in HIV/AIDS patients in Nigeria with less pathogenesis among non-HIV individuals hence, $P < \pm 1.96$ critical region, we accept that, there was a significant difference in the distribution of Microsporidiosis among sexes in Makurdi with the $\chi^2 = 0.59 < 7.8$ at 5% level of significance (indicating a significant difference in sex related prevalence at the Jos, Plateau). However, there was high level of social behavior observed at Makurdi with a general low CD4 counts (<50 cell/ μl-<100 cell/ μl).

Keywords: Pilot. Microsporidiosis. CD₄. T- cells. North-central.

Introduction:

Microsporidia are widespread small unicellular obligate intracellular parasites which several of these protozoan parasites of the Phylum-Microspora are now being detected in humans with frequency in patients with human immunodeficiency virus (HIV). Microsporidia is a protozoa that belong to the class-Microsporea which presently is known to cause several disease conditions in HIV, as well in malnourished individuals. They are protozoan parasites of the intestine, muscle, cornea, liver and several other tissues of fish and reptiles. Many invertebrates groups especially insects are infected essentially in all tissues (Canning and Hollister, 1987). In the case of HIV causative agent of AIDS, their pathogenic effect have become outstandingly important because of their increased pathology, unlike some other several protozoan parasites, microsporidia have rarely been studied because of the diagnostic problem due to their size and the invasive nature of the specimen collection from infected subjects (Canning and Hollister, 1987; Bryan *et. al.* 1994).

Moreso, Microsporidiosis have become an important opportunistic infection in immunocompromised persons infected with human immunodeficiency virus (HIV), microsporidial infection are increasingly a significant course of morbidity and high mortality rates in AIDS infected persons. Two microsporidian genera; *Encephalitozoon* sp. is known to cause ocular disease, hepatitis and peritonitis. More frequently intestinal infections are associated with *Enterocytozoon bieneusi* (Canning and Hollister 1987; Terada *et. al.* 1987; Zender *et. al.* 1989; Bryan *et.al.* 1990; Cali *et.al.* 1991). Microspordia infections is now been recognized in Jos and Makurdi as an indigenous public health problem and most other part of the world. This will pose a challenge to health workers and the general public in Nigeria, because the number of susceptible, potential human host for these parasitic infections will continue to increase in the coming years if not checked. There appears to be a systemic association between Microsporidial involvements of the eyes, urinary tract and bronchial tree, any persons will have symptoms referable to the kidneys, bladder or lungs (Bryan *et. al.* 1994). However, the spectrum of the illness associated with AIDS has been enlarging since its initial description in 1981 and the gastrointestinal tract continues to be one of the major targets of this devastating disease. The many causative agents include protozoa's, helminthes and probably various non infectious diseases which have not been discussed in this research finding. The aim is to isolate microsporidium species as opportunistic infection among the study subjects at Jos and Makurdi community of Plateau and Benue State, Nigeria.

Materials and Methods

Study Area

Jos and Makurdi in Nigeria lies on the pre-Cambrian to the Cambrian and Jurassic Northern Nigeria crystalline complex. It covers about 9,400km² of the crystalline complex in central Nigeria. Its average elevation is about 1,250m above mean sea level. The land surface generally consists of plains, hills, depression and rocks of various forms, shapes and sizes. Jos the Plateau State remains the major tourist center in Nigeria.

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Methods

Assay Procedure

Fecal specimen of 10g were homogenized in 10ml of saturated NaCl, filtered through a mesh gauze of 30, 60, 90 and centrifuged at 200g for 10 minutes. 100 μ l sample of the top layer were removed and wash twice in distilled water by centrifugation at 1000rpm for two minutes, and were stained in 10% giemsa for 30 minutes, it was washed with distilled water or running tap and allowed to dry. Oil immersion was used to view the slides using a light microscope x1000. Microsporidia test procedure was adapted (Canning and Hollister, 1987; Weber *et.al.* 1992).

Coulter® Manual CD₄ Counts Kit

Blood samples of sufferers were collected into EDTA containers after informed consent and ethical clearance from a research ethics committee. This assay depends on the ability of monoclonal antibody coated latex spheres to bind to the surface of cells expressing discrete antigen determinants. When the CD₄ coated latex spheres come in contact with a cell that has the CD₄ cell surface antigen, the two bind forming a cell- latex spheres rosette that is readily

recognized by light microscopy. The size of the CD₄ coated latex spheres was measured from 1.8 to 2.2 microns in diameter, which represents 0.9 to 2.2% of the depth of most hemocytometers. CD₄ coated latex spheres also react with monocytes. Absolute CD₄ lymphocyte counts are performed usually after combining an aliquot of whole blood with MY₄ cyto-spheres monocyte blocking reagent adding the CD₄ cytospheres reagent to lyse erythrocytes. Crystal violet, a component of the lysing reagents, is used to stain the nuclear material of the leucocytes to facilitate identification in hemocytometer. However, manufacturer's recommendations were strictly followed as a progressive clinical and immunologic deterioration generally correlates with a falling CD₄ lymphocyte count.

Results interpretation

Intestinal Tract

Serious illness of microsporidial infections was seen predominantly in adults suffering from immunosuppression, associated with HIV/AIDS. Substantial evidence of *Enterocytozoon* spp. and *Encephalitozoon* spp. was established among the 500 and 2008 specimens collected at the Jos Plateau and Makurdi respectively (Plate I).

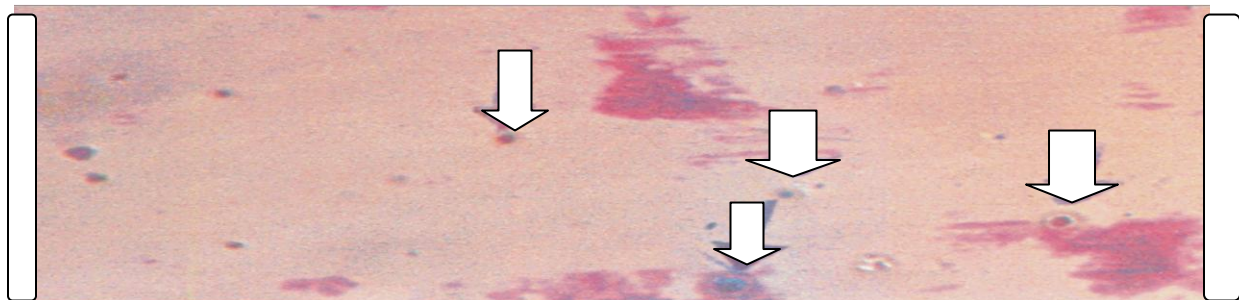


Plate 1: Parasitological Diagnosis of Positive *Ent. bienewisi* from human faeces. Magnification x 1000

The preliminary isolation of microsporidium spp. in different category of persons under examination, and apparently individuals were without microsporidium in both sexes and the CD₄ counts >350 cell/ μ l (Figure 1). Gastroenteritis 1(0.5%) female had microsporidium with CD₄ count <350 cell/ μ l. Others include 7(14.0%) males, 8(16.0%) females of HIV individuals with pathogenic microsporidiosis and CD₄ counts <200 cell/ μ l against 12(24.0%) males, 7(14.0%) female AIDS patients with deterioration of CD₄ <100 cell/ μ l. However, in both sexes, the males had the highest infection rate with low risk factor of pathogenesis. There was high rate of infection among the acquired immunodeficiency syndrome (AIDS) individuals with significant difference in the categories of persons examined, given that $\chi^2=0.59 < 0.05\%$ level of significance. Microsporidiosis was a serious threat to AIDS patients which reveals serious profuse diarrhea with emaciation (5-10times bowel/day) lasting 2-3 months after which the persons condition gets worsening (Table 1, figure).

Table 1: Preliminary CD4 counts among individuals with microsporidium infection at the Jos Plateau, Nigeria.

Category of persons examined.	Sex		Total No. examined	No. positive (%)	CD4 T-cells count/ μ l.
	No. of males examined	No. of females examined			
Apparently Healthy individual	50	0(0.0%)	50	0(0.0%)	>350 cell/ μ l
Gastroenteritis	100	0(0.0%)	100	1(1.0%)	<350 cell/ μ l
HIV	50	7(14.0%)	50	8(16.0%)	<200 cell/ μ l
AIDS	50	12(24.0%)	50	7(14.0%)	<100 cell/ μ l
Total	250	19(7.6%)	250	16(6.4%)	35(7.0%)

HIV= Human immunodeficiency virus. **AIDS**= Acquired immunodeficiency syndrome. **No.** = Number

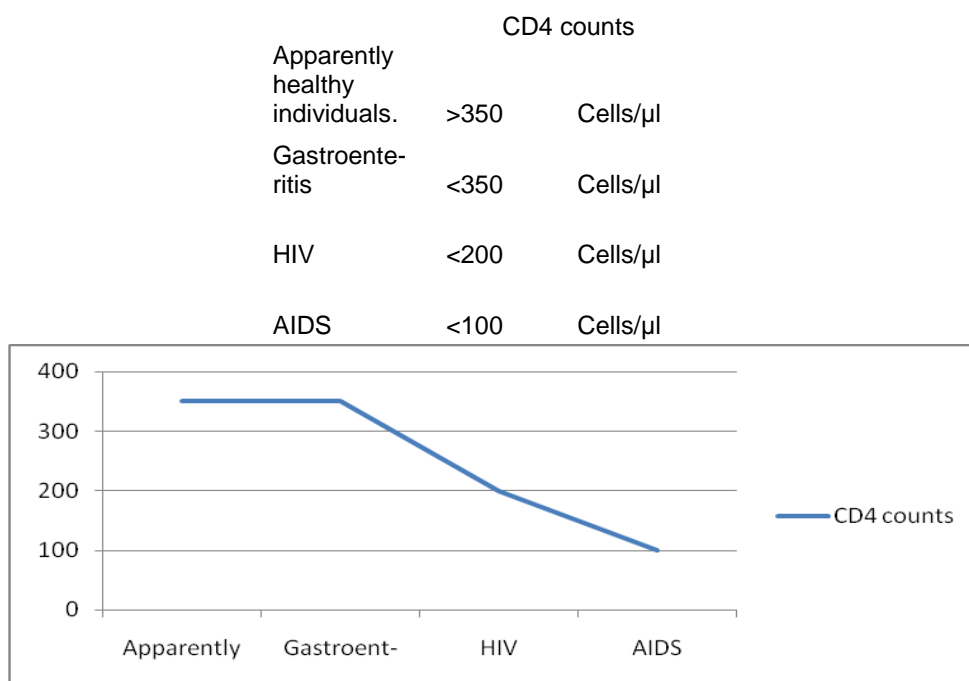


Figure I: Cells/ μ l of CD4 counts among categories of individuals infected with microsporidium spp. in Makurdi, Benue State, Nigeria.

The intestine became involved in some cases of *Enterocytozoon bieneusi* though, from a view point, this microsporidian is invasive and infecting the enterocytes. The isolation of microsporidium species shows in table 2, figure II where the total sum of *Enterocytozoon bieneusi* and *Encephalitozoon cuniculi* infection were 156/5(3.2%) for students followed by the Civil Servants 634/36(5.7%) with the CD₄ <100 cell/ μ l (Figure II). Housewives were

499/44(8.8%) and CD₄ counts <50 cell/ μ l and other (non-categorize group) were 719/11(1.5%) with CD₄ cells <90 cell/ μ l. Isolated *Enterocytozoon bieneusi* were high (3.0%) against 1.8% of *Encephalitozoon cuniculi* prevalence rate in all the categories examined. Since $P < \pm 1.96$ at a critical region, we accept therefore that, there was a significant relationship in the distribution of microsporidiosis in all the categories of persons examined.

Table 2: Parasitological isolation of microsporidium species among HIV/AIDS patients at Makurdi, Benue State, Nigeria.

Category of persons examined	Microsporidium species				Total No. sampled	Total No. infected (%)	CD4 cells count.
	No. positive with <i>Enterocytozoon bieneusi</i> (%)		No. positive with <i>Encephalitozoon cuniculi</i> (%)				
Students	3	(1.9%)	2	(1.3%)	156	5 (3.2%)	<100 cell/ μ l
Civil servants	20	(3.2%)	16	(2.5%)	634	36 (5.7%)	<100 cell/ μ l
Housewives	26	(5.2%)	18	(3.6%)	499	44 (8.8%)	<50 cell/ μ l
Others	11	(1.5%)	0	(0.00)	719	11 (1.5%)	<90 cell/ μ l
Total	60	(3.0%)	36	(1.8%)	2008	96 (4.8%)	

No. = Number

Others = Those without a definite occupational status.

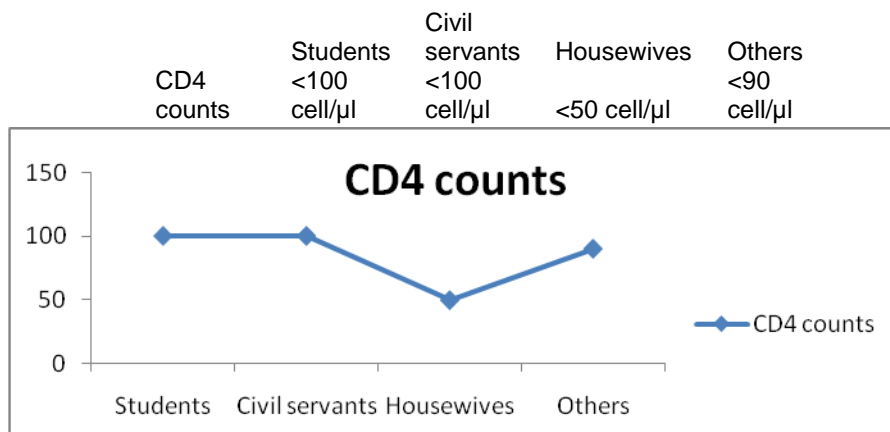


Figure II: Cells/ μ l of CD4 counts among categories of individuals infected with *microsporidium* spp. at Jos, Plateau State, Nigeria.

Urogenital Tract

Urinary tract microsporidiosis appears to be common in patients with *Encephalitozoon* symptoms referable to the kidneys or bladder and probably the urinary tract may be the less affected organ system. Infections, although few persons do not showed

Discussion

At least two cases of severe microsporidial disease have been observed in HIV/AIDS patients and in other category of individuals examined. Intestinal infections with *Enterocytozoon bieneusi* manifested in HIV/AIDS patients with chronic diarrhea, anorexia and a weight loss. Patients typically report from 5 to 10 times bowel movement/day (ranging from 1 to more than 15). The stools were watery and loose due to invasive nature of the intestinal pathogens, and none bloody as may defly in some report without fecal leucocytes. Immunologic countdown showed in some quota and gradual deterioration of cells mediated immunity. Report however, of deaths directly attributable to *Enterocytozoon bieneusi* was never recorded in this case study but reported mortality rates in patient's with intestinal *Enterocytozoon bieneusi* was recorded as high as 56% (Pol *et.al.* 1993). As with *Enterocytozoon*, CD₄ counts were generally low in AIDS patients with encephalitozoonosis. In addition, the spectrum of recognized *Encephalitozoon-*

associated includes nephritis, ureteritis, hepatitis, fullminant hepatic failure, and peritonitis (Weber *et. al.* 1994; Schwartz *et. al.* 1997). However, it was similarly observed that, *Encephalitozoon*-like microsporidian was associated with the general skin infection though, *Encephalitozoon* infections may exhibit a wide range of severity so much that, laboratory evidence for intestinal malabsorption was observed common in AIDS with CD₄ lymphocytes count very low (<100 cells/ μ l) and this coincide with the report of Orenstein (1991). Here, it was a concern that, a protracted debilitating illness such as HIV/AIDS severely impact on the person's quality of life and prolonged diarrhea up to 4 months was attributed to the illness. Nevertheless, other symptoms associated to *Enterocytozoon bieneusi* which was observed among fewer of the AIDS patients include abdominal pain, nausea, vomiting and high fever with accompanied headache. However, these findings represented a medical manifestation consistent with the *Enterocytozoon bieneusi* which is now recognized at the Jos, Plateau and Benue State a leading cause of AIDS-related peritonitis and probably hepatitis as often revealed from many reports on the bile duct, intestinal, gall bladder abnormalities. Data to support perfect preventive measures are quite limiting to the presence of infective spores in body fluids and faeces, attention should be drawn to health care, and other institutional setting to strongly advocate on

meticulous hand washing which will help in preventing primary infections, as well other hygienic conditions. Besides, co-habiting sexual partners of infected patients should be offered screening for microsporidiosis regardless of their HIV status.

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