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OCCURRENCE OF INTESTINAL MICROSPORIDIA INFECTION AMONG HIV/AIDS PATIENTS ATTENDING GENERAL AND DIVINE MERCY HOSPITAL MINNA, NIGER STATE

[1]Ubanwa, E. D[1]. Nnamani, S. M.[2] and Benjamin, G, Y[1]

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Abstract

Microsporidia infection may be asymptomatic in immunocompetent individual; however, can be severe and disseminated among HIV/AIDs patients, children less than five years, elderly, immunocompromised patients. Of 748 samples was collected from HIV - and HIV/AIDS tested for microsporidia using Giemsa stain, 11(1.47%) were positive. Stool hard 9(2.14%) and urine 2(1.01%) all from HIV/AIDS while HIV - show no positive cases at $p < 0.05$ a significant difference was recorded. Age and sex, highest was within 26 - 35 years (3.29%) female hard highest (1.84%). Predisposing factor, cases of hard (5.48%), while pipe born water (0.00%). It has proved that the parasite is highly associated with diarrhoea and among immunocompromised patient as an opportunistic infection. Immunocompromised patient, elderly, and organ transplant individual have to improve on both personal and environmental hygiene.

INTRODUCTION

Microsporidia are intracellular groups of parasites that have attracted the interest of parasitologists, for over centuries of years. The first species of microsporidia to be discovered was *Nosema hombycis*, in the middle of the 19th century as the cause of silkworm disease (i.e., pepper disease, pehrine disease), which nearly destroyed the silkworm industry in Southern Europe (Didier, 2005; Franzen, 2008).

The first report on human microsporidia infection was in 1957, when a case of a 9-year-old Japanese boy who presented with disseminated Microsporidiosis associated with fever, headache, vomiting, and spastic convulsions (Matsubayashi *et al*., 2016). Human intestinal Microsporidia are small (1 to 2 mm), parasites characterized by a polar filament that is extruded during the invasion of the host cell (Didier, 2005). Mature Microsporidia spores

[1]State University of Medical and Applied Science (SUMAS)
[2]Enugu State University of Science and Technology (ESUT)

have thick three-layered walls and can pass through some water treatment filters due to their small size also resistant to chlorine at concentrations used in treating drinking water. Microsporidia spores have been found in drinking water sources, soil, and domestic and wild animals; suggesting the possibility of water-borne, food-borne, zoonotic, and anthropologic transmissions (Ubanwa *et al*., 2018). The species of this parasite comprise a diverse phylum of obligate intracellular parasites consisting of approximately 150 genera and more than 1300 identified species (Le, 2010). Only about 7 genera and 15 species have been associated with human infections (Franzen, 2008; Didier *et al*., 2005; Weiss, 2001). The pathways of microsporidia infections, modes, or routes of transmission and the knowledge of the epidemiology are still uncertain. This is because the parasite spores are released into the environment via stool, urine and respiratory secretion. Possible source of infection may be person or animal infected with microsporidia (Dworkin, *et al.,* 2007)

Interest in this group of parasites started since advent of Acquired Immune Deficiency Syndrome (AIDS) pandemic around the world in 1980's. In 1985, a new species *Enterocytozoon bieneusi* was found in Human Immune Virus (HIV) infected patient (Desportes *et al*., 2015). Two species, *E. bieneusi* and *E. intestinalis* have discovered to be responsible for the majority of the gastrointestinal and biliary tract infections in humans. Since then, species of microsporidia have been recognized worldwide as etiologic agents of opportunistic infections. The clinical case depends on the immune status of the patient and the site of infection. The group at risk constitute HIV/AIDS patients, organ transplant recipients being treated with immunosuppressive drugs, travelers, children and the elderly (Garcia, 2002; Dworkin *et al*., 2007; Galvan *et al*., 2011; Wichro *et al*., 2005; Rabodonirina *et al*., 2003). The purpose of this study was to determine the occurrence of intestinal microsporidia infection and its associated risk factors among HIV/AIDS patients in Minna, Niger state, Nigeria.

MATERIALS AND METHODS

Study Area and sites

The study was conducted in Minna, Niger state. Niger State lies on the 3.20° East and longitude 11.30° North with land mass covered area of 74,244 square kilometers, which is 8% of the total land area of Nigeria. Two study sites were chosen; General and Divine Mercy Hospital respectively in Minna, Niger state.

Study population and sample collection.

HIV/AIDs patients was recruited in this study, 748 samples from 460 participants, stool 502 and 246 urine samples. Each patient was giving a stool sample container label with his or her hospital card number for proper identification. A structured questionnaire, was used to collect data on demographics.

Stool Examination

Approximately 0.5g of stool was homogenized in 10ml of distil water in a test tube; the homogenate result was filtered through a 300 µm mesh sieve into a centrifuge tube and centrifuge at 2000 rpm for 10 minutes. The supernatant was pipette and discarded, the sediment was washed twice in a distilled water for 2 minutes at 65000 rpm, and the pellet was deposited in 15ml distilled water thereafter centrifuge at 1000 rpm for 2 minutes. The resultant sediment was used to make smear on a clean glass slide, air dry and fixed in methanol for 1 minute, stained with 10% Giemsa solution and raise in gentle running tap water allowed to dry and view at X 100 magnification oil immersion (Ubanwa *et al*., 2018).

Urine Examination

Urine sample (5ml) from sample bottle were discharge into centrifuge tube and centrifuged at 12500 rpm for 10 minutes, the supernatant was discarded and the sediment (pellet) was washed in a micro centrifuge at 65000 rpm for 2 minutes, the resulted crude was re-suspended in Phosphate Buffered Saline (PBS) and centrifuged in a cytospin centrifuge at 1000 rpm for 2 minutes. The end product was used to make smear on a clean glass slide air

dried fixed in methanol for 1 minute stain with Giemsa solution and raise in gentle running tap water allowed to dry and examine at X 100 magnification oil immersion (Ubanwa *et al*., 2018).

Questionnaire

A questioner was design to determine some common factors that predisposed man to Microsporidia. Questions covers among others: Environmental sanitation and living condition characteristics (i.e. types of water supply, latrine system, sewage disposal system, and presence of domestic animals); Any case of diarrhea before or now; Any case of symptoms of gastroenteritis (Hutin, *et al*., 2008).

Ethical Clearance, Participants Information and Consent Form

Ethical clearance was approved by Ethical committee Federal University of Technology and General Hospital Minna, Niger state, and all works were performed according to the guidelines for human experimental in clinical research stated by the Federal Ministry of Health Nigeria. Participant's information leaflet was submitted along with application for Ethical clearance and was conceded. Consent form were read and translated to the hearing of participant and they accepted to participate in this research by endorsed it.

Data Analysis

The prevalence data was computed in percentage and subjected to chi square analysis to-illustrate relationship between the subject studied and among the study area. The analysis was assumed significant at $p < 0.05$ level of significant.

RESULTS

The prevalence of microsporidia infection among HIV/AIDS patients attending general hospital Minna was 1.47% out of 748 samples of both stool and urine. In this study stool hard the highest prevalence of (2.14%) among the HIV/AIDS participants, while HIV - participants that serve as control no positive sample was recorded. In urine sample (1.01%) was recorded among HIV/AIDS participants. There was a significant difference within the samples screed and subject participant at $p < 0.05$.

Table 1. **Prevalence of microsporidia in HIV/AIDS Patients in General Hospital Minna**

Sources of samples	Overall No. Exam + (%)	HIV - No. Exam + (%)	HIV/AIDS No. Exam + (%)
Stool	502 9(1.79)	82 0(0.00)	420 9(2.14)
Urine	246 2(0.81)	48 0(0.00)	198 2(1.01)
Total	748 11(1.47)	130 0(0.00)	598 11(1.83)

χ^2cal. 1.96; χ^2tab. 12.59, df. 2; $p > 0.05$

Age and Sex prevalence of microsporidia infection shows highest prevalence was recorded within the age 26 - 35 years old (3.27%) among HIV/AIDS, while least was found at the age of 0 - 14 and 36 - 45 years old respectively. In HIV - no positive cases was recorded hence it serves as control. With respect to Sex the highest prevalence was recorded in female HIV/AIDS participants (1.84%) while male hard (1.83%), although there was no significant difference within the sex and age at $p < 0.05$.

Table 2. Sex and age prevalence of Microsporidia infection in Immunocompromised Patients in General Hospital Minna

	Sex			
	HIV - (control)		HIV/AIDS	
	Male	Female	Male	Female
Age	No. Exam. + (%)	No. Exam. + (%)	No. Exam. + (%)	No. Exam. + (%)

0 - 14	7	0(0.00)	11	0(0.00)	41	0(0.00)	62	0(0.00)
15 - 25	10	0(0.00)	16	0(0.00)	52	1(1.92)	84	2(2.38)
26 - 35	8	0(0.00)	23	0(0.00)	61	2(3.27)	91	3(3.29)
36 - 45	13	0(0.00)	18	0(0.00)	30	0(0.00)	76	1(1.31)
46 +	8	0(0.00)	16	0(0.00)	34	1(2.94)	67	1(1.49)
Total	46	0(0.00)	84	0(0.00)	218	4(1.83)	380	7(1.84)

χ^2 cal.6.464; χ^2 tab: 6.86; df: 12; $P < 0.05$

Some predisposing factor to microsporidia shows that cases of diarrohea hard (5.48%) prevalence rate infection, while those without any case recorded (0.65%). Participant that disposes their waste close the house hard (4.28%) and government approved area (2.08%). Defecation variable shows those uses bushes hard (2.71%), while water system toilet (2.32%) and pit toilet (2.02%). Domestic water supply, pipe born (0.00%), boreholes (3.48%), wheel (1.70%) and Stream/River (5.00%). Presence of domestic animal: Yes hard (2.06) and No (2.55%). There was a significant difference within the source of water at $p < 0.05$.

Table 3. Potential risk factors associated with microsporidia infection in Minna

Variables	No. Exam.	No. +ve	Infection (%)	95% CL	P. value
Sources of water					
pipe born	28	0	(0.00)	7.815	51.247
Boreholes	86	3	(3.48)		
Wheel	294	5	(1.70)		
Stream/River	60	3	(5.00)		
Defecation					
Pit toilet	198	4	(2.02)	5.99	0.099
Water system	86	2	(2.32)		
Bushes	184	5	(2.71)		
Waste disposal					
Govt. approved area	96	2	(2.08)	3.841	0.291
Close to house	210	9	(4.28)		
Presence of domestic animal					
Yes	194	4	(2.06)	3.440	0.639
No	274	7	(2.55)		
Cases of diarrhoea					
Yes	164	9	(5.48)	3.921	0.732
No	304	2	(0.65)		

DISCUSSION

Microsporidiosis in human occurs worldwide but prevalence vary widely depending on the geographical region, population studied and diagnostic methods used. Prior to the AIDS pandemic, Microsporidiosis was rarely identified in humans (Abba *et al.*, 2011). Prevalence data for microsporidia in human populations before the era of AIDS relied upon serology based on detecting antibodies to *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis* and or *Encephalitozoon cuniculi*, the species in mammals. Seroprevalence result ranged from 0 to 42%, with the highest rate found in homosexual men in Sweden and in person with other parasitic infection (Hollister *et al.*, 2013). A wide range of case reports have identified microsporidia in non -HIV infected individuals but prevalence data based on parasite detection (versus serology) are inadequate (Bryan and Schwartz, 2013). Modified Giemsa staining technique

for stool provides a useful means of screening clinical specimen, the occurrence of microsporidia in immunocompromised individual and immunocompetent conforms with earlier report that microsporidia occurs mostly .among immunocompromised individual particularly HIV/AIDS patient (Hollister *et al*., 2013).

In the study an overall prevalence was (1.47%) which is in agreements with Hollister *et al*. (2013). In stool samples (2.14%) was recorded from all groups of both HIV - and subjects investigated. This conforms to earlier study by Sokolova *et al*. (2011) who reported that one specie of microsporidia (*E. bieneusi*) in over 68% of unexplained cases of diarrhoea involving AIDS patients. Sulaiman *et al*. (2013) most common microsporidia found in man is *E. bieneusi* causes intestinal infection among patients with AIDS and diarrhoea.

This present study showed that the prevalence is more in female than male counterpart, this is the fact that females participants were more in the in study population, also visits hospitals for regular checkup and more exposed themselves to some potential risk factors that predisposed men to microsporidia infection. This report is in conformity with study of Oladele *et al*. (2015) who stated that woman are the main childcare providers and housekeeper in Nigeria that this were probably responsible for the high occurrence of both cryptosporidium species and E. bieneusi in them. Woman in general has more frequent contacts with children or animals than men. Although no significant difference in the prevalence of microsporidia according to gender of the participants and this is consistence with the result of previous report of Lono and Kumar, (2010). On the other hand the current study reported significantly higher odds of microsporidia infection among aged of 25 years as compared to younger individuals. This in line with study conducted by Tengku *et al*. (2013) who recorded 1.97% infection rate in Malaysia in > 15 years. Similarly, Norhayade *et al*. (2008) reported a high prevalence of 57.2% of microsporidiosis among adult aged more than 31 years. The high prevalence rate of the infection in this study among the > 15 years might be explained by the fact that their behavior is related to more active movement and more independent eating habits compared with children.

The observations that microsporidia are ubiquitous in nature suggest that several modes of transmission and sources exist for human infections. The possibility of human-to-human transmission cannot be ruled out. Experimental examples of microsporidia transmission between laboratory animals might suggest that the same infecting species could be transmitted between humans via the same pathways as in animals (Bennuru, *et al*., 2005). This study analyzed risk factor reveal that the presence of other family members infected with microsporidia increases the odds for acquiring microsporidiosis by 8 times. In addition, the research were able to identify several significant risk factors for infection: the household presence of and contact with droppings of domestic animals (especially ducks and chickens); lack of a flush toilet, municipal garbage collection, and running water. Some of mild infections may have missed because only microscopy was used as our primary screening tool; PCR would have been more sensitive, but may be impractical for screening the large number of specimens analyzed in the study. Likewise, a study done by Leelayoova *et al*. (2005) suggested that human-to-human transmission occurred in an orphanage, where multivariate analysis showed that orphans who were 12-23 months old and living in one particular house were independently associated with *E. bieneusi* infection and all infected children presented the same genotype in a stool samples. Individuals with a history of contact with diarrhoea patients had two times greater risk of getting the microsporidia infection (Gumbo *et al*. 2011). Several questions still exist about clinical aspects and consequences of microsporidia infection in humans. In general, the clinical course of microsporidiosis depends on the immune status of the host and site of infection (Didiere *et al*., 2005).

CONCLUSION

It is obvious the microsporidia infection occur among the studied HIV/AIDS participants, it is important that both personal and environmental hygiene of the participant should be encourage either through advertisement in public place or jingle in radios. It is advise that diagnoses of microsporidia and other opportunistic infection parasite should be included in their rooting check of this group of patient.

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