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Research Article

Current Status of Microsporidium among Hospitalised Human Immunodeficiency Virus (HIV/AIDS) Infected Patients, Federal Medical Centre, Keffi, Nigeria

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Abstract

Microsporidia are obligate intracellular parasitic fungi causing chronic diarrhea, particularly among immunocompromised patients. Microsporidial infections have been recognized as an increasingly important infection, particularly among those hospitalised with HIV/AIDS infected patients at the Federal Medical Centre (FMC), Keffi, Nasarawa state. One health facility was used to engage HIV/AIDS infected patient accessing Antiretroviral Therapy (ART) to identify the presence of *microsporidium* and to determine the associated risk factors. 252 stool samples were examined for microsporidial spores by modified giemsa staining technique. The overall prevalence rate of 15.08% was recorded. Based on age group, microsporidia infection was common among patients aged between 61-70 years 15.0% while sex related, the male had 30.43% rate of infection. Though, marital status, occupation, and widow/widower had 15.79% and artisans (33.33%) respectively. The vulnerability was determined by their fate and status. However, *chi-square* result showed no significant relationship observed (P>0.05) between the age, and sex distribution of microsporidium. The proportion among occupation and marital related distribution of microsporidium among HIV/AIDS infected patients were diarrhoic. In relation to viral load, all positive HIV/AIDS infected patients with microsporidia spore had viral load above normal. Twenty-five (25) HIV/AIDS infected patients had 10,000 ml/viral load in replicates. Microsporidium is therefore, identified and recognized as an invasive opportunistic infection among HIV/AIDS infected patients which should be considered in a routine checks among HIV/AIDS infected patients.

Keywords: *Microsporidia*, HIV/AIDS, Microsporidium, Infected patients, Federal Medical Centre (FMC)

INTRODUCTION

Microsporidia, classified as highly specialized fungi, are unicellular and obligate intracellular opportunistic pathogens which can infect a wide range of vertebrate and invertebrate hosts such as fish, insects, farm and companion pets (Han et al., 2017), and (Li et al., 2019). The phylum *Microsporidia* is consisting of more than 170 genera and 1300 species. Among these genera, eight of them have been responsible for human infections, including *Enterocytozoon*, *Pleistophora*, *Encephalitozoon*, *Vittaforma*, *Trachipleistophora*, *Brachiola*, *Nosema* and *Microsporidium* (Ghoyounchi, 2017), (Didier ES et al., 2005), and (Anane S et al, 2010). *Enterocytozoon bieneusi* (*E. bieneusi*) and the *Encephalitozoo*n species (*E. cuniculi*, *E. intestinalis* and *E. hellem*) are the four major species infecting humans. *E. bieneusi* which is responsible for more than 90% of cases with microsporidiosis in humans is most commonly diagnosed (Deng L et al., 2017) and (Matos O et al., 2012).

Microsporidial infections have been reported to occur in severely immunocompromised individuals, mainly HIV/AIDS patients, but cases in HIV negative people, including travellers and elderly people, are continually

increasingly recognised worldwide as opportunistic infection agent. (Didier, et al., 2011) Of the several species that infect man, *Enterocytozoon bieneusi* were the first documented case and the most commonly recognised *Microsporidia* that caused gastrointestinal disease in immunocompromised patients particularly in HIV/AIDS patients.

This parasite is commonly observed in HIV infected patients with high viral load of 10,000 ml and CD4 lymphocytes count of less than 50 cells/mm^3 who complain of chronic diarrhoea, nausea, mal absorption and severe weight loss. Whereas *Encephalitozoon intestinalis* causes both a disseminated and intestinal infections associated with nephritis, sinusitis or bronchitis (Daar ES, 2021). As a zoonotic pathogen, the main transmission way of *E. bieneusi* is faecal-oral route or oral-oral route because its spores are shed into environment *via* faeces. Therefore, the way of consumption of contaminated food and water is the main route of *E. bieneusi* infection, this will help determine current status of microsporidium in Human Immunodeficiency Virus infected patients attending Federal medical center, Keffi.

MATERIALS AND METHODS

Study area and population

The study area this research was conducted among 252 consenting HIV/AIDS positive patients cover the period in 2021 at the Antiretroviral Therapy (ART) clinic of the Federal Medical Centre (FMC) Keffi, Nasarawa state, Nigeria. The general topography of Nasarawa state is that of hills/dissected terrain, undulating plains and lowlands. The state has a climate typical of the tropical zone with a maximum and minimum temperature of 81.7°F and 16.7°F respectively with moderate rainfall which varies from 131.73 cm in some places to 145 cm in others. The state has a total land area of 27,117 km^2 (10,470 sq mi) and a population of 1,869,377 according to 2006 census and the main economic activity is agriculture (Figure 1).

Study design

This study was a cross-sectional study which includes both male and female HIV/AIDS hospitalised infected patients at the federal medical centre, Keffi, Nasarawa state Nigeria. Consent was sought among infected individuals and stool samples were collected and examined. The viral load of HIV/AIDS patients were taken between the periods of study 2021.

Sample collection

About 10 g of fresh stool samples were collected from each individual HIV/AIDS patients and each sample were well labelled with the encryption of the patient's identity; sex, age, occupation and date of collection within 14 days. A clean dry, mouthed sample container with 10% formalin saline was given to each person's for collection of stool sample and stored at room temperature until it was processed.

Method

Giemsa stain method: The slides were prepared from homogenized stool samples and one drop of methanol onto the slide was fixed and was allowed to dry for 1-2 minutes. The slides were stained in 10% giemsa for 1 hr and was washed with tap water and allowed to air dry. Slides were examined using x 100 magnification (oil immersion). Giemsa stained spores were oval, with the cytoplasm staining light grey blue with a dark stained nucleus spores.

The versant HIV-1RNA 3.0 bDNA system tests was used for direct quantification of type-1 human immune-deficiency virus in plasma from individuals with HIV by amplification of the signal emitted by the nucleic acid, using a buyer system 34 bDNA analyser was used for viral load test (Eligio L, et al., 2016).

Data analysis

The statistical package was used for data analysis. Prevalence of infection was given in percentages in line with the variables. *Chi-square* and 95% confidence interval analysis were the statistical tools used to determine significance at a cut off value of 95% (p=0.05).

RESULTS

Age and the sex related distribution of microsporidium among HIV/AIDS infected patients.

The two hundred and fifty two (252) HIV/AIDS examined, were patients hospitalized and their stools sampled for examinations thus, 38 HIV/AIDS persons among the large number examined were identified/established to be affected with microsporidium spp. between the ages of 11-20 years in both sexes, 2 (12.50%) were positive with the high number 10 (11.90%) of HIV/AIDS individuals suffering from the prolific diarrhoic microsporidium particularly among females aged between 31-40 years and 41-50 years 16(20.00%), availing the state of vulnerability in the age category, and between aged 61-70 years, 2 (66.67%) were infiltrated with microsporidium among the least most infected in the age group considered vulnerable with no significant relationship in the infection of microsporidium among the ages and sexes of the infected AIDS patients. (X^2=6.10<11.07, df=5, P<0.05) (Table 1).

Table 1. Age and sex related distribution of *microsporidium* among HIV/AIDS infected patients.

Sex					
	Male		Female		Total
Age group	No. examined (%)	No. positive (%)	No. examined (%)	No positive (%)	No positive for both sexes
11-20	4	2 (50.00)	12	0 (0.00)	2 (12.50)
21-30	8	4 (50.00)	18	4 (22.22)	8 (30.77)
31-40	11	0 (0.00)	73	10 (13.70)	10 (11.90)
41-50	20	6 (30.00)	60	10 (16.67)	16 (20.00)
51-60	0	0 (0.00)	42	0 (0.00)	0 (0.00)
61-70	3	2 (66.67)	1	0 (0.00)	2 (50.00)
Total	46	14 (30.43)	206	24 (11.65)	38 (15.08)

Marital status and sex-related distribution of microsporidium among HIV/AIDS infected persons were very much determined with the status of their viral load as descrepancies can be seen among the infected individuals so much that widows/widowers of both sexes 6 (15.79%) had viral load between 520 ml-56,600 ml in contrast to the value/ml volume of the viral load among the married with 56,300 ml-3,900 ml, as well among the singles (56,600 ml-92,300 ml) this, clearly showed the status and the potency of the immune system among group of individuals, the higher the viral load, the lower the CD4 counts which means the state of vulnerability among the infected HIV/AIDS patients was simply established (Table 2).

Table 2. Marital status and sex related distribution of microsporidium among HIV/AIDS infected patients.

Sex					
	Male		Female		Total
Marital status	No examined (%)	No positive (%)	No examined (%)	No positive (%)	No positive for both sexes
Widow/Widowers	12	4 (33.33)	8	2 (25.00)	6 (15.79)
Married	22	4 (18.18)	119	14 (11.76)	18 (12.77)
Singles	12	6 (50.00)	79	8 (10.13)	14 (15.38)
Total	46	14 (30.43)	206	24 (11.65)	38 (15.08)

The prevalence of microsporidium spp. among the occupation and sex related HIV/AIDS infected person was obvious, considering the occupation of the individuals that mostly exposed them to such infection, particularly famers who by the nature of tiling the land, are always accessible and most vulnerable to the soil in their dried muddy/loamy state. Farmers in both sexes 6 (25.00%) were positive with microsporidium with their viral load 513-92,300 ml as it differs among individuals for student category, 14 (15.38%) were stigmatized with the viral load between 20-56,600 ml meaning that, the low their CD4 counts in contrast to the civil servants 16 (12.31%) with viral load 15 ml-1.710 ml which differ with the higher viral load in the unemployed who most are left with no choice to life and among the unemployed 1 (25.00%) had over 1,890 ml-58,400 ml with the artisans 2,590 ml-319,000 ml given a wide margin among the categories in the structure though, the proportion of the infection was relatively significant to the status of the viral load (Table 3).

Table 3. Occupation and sex-related distribution of microsporidium among HIV/AIDS infected patients.

Sex					
	Male		Female		Total
Occupation	No examined (%)	No positive (%)	No examined (%)	No positive (%)	No positive for both sexes
Farmers	10	3 (30.00)	14	3 (21.43)	6 (25.00)
Students	9	3 (33.33)	82	11 (13.41)	14 (15.38)
Public/civil servants	23	7 (30.43)	107	9 (8.41)	16 (12.31)
Unemployed	2	0 (0.00)	2	1 (50.00)	1 (25.00)
Artisans	2	1 (50.00)	1	0 (0.00)	1 (33.33)
Total	46	14 (30.43)	206	24 (11.65)	38 (15.08)

However microspridium related viral load among HIV/AIDS showed that, more of the suppressive tendencies of the virus can be expressed by simply silencing the ability of the immune capacity of the

individual whose condition seems deteriorating, meaning that the CD4 counts showed no capacity to replicate hence, stagnation of the immune system which would have assume to boost the status of the patients health was due to negligence and lack of adherence strictly to antiretroviral drugs treatment. Of the 234 HIV/AIDS patients, 8.55% had less viral/ml volume in contrast to 10 persons with HIV/AIDS who had 100% viral load between 10,000 ml-30,000 and 8 HIV/AIDS with 30,000 and 319,000 ml higher per value/ml volume and very low was their CD4 counts exhibiting more clinical symptoms HIV/AIDS full blown vulnerability, at this time most patients were passing out putrid diarrhoea, pale with unsteady movement, blisters of the mouth lips and other undesirable symptoms (Table 4).

Table 4. Microsporidium and viral load among HIV/AIDS infected patients.

Viral load range	No. of HIV/AIDS patients	No positive with microsporidum spp(%)
20 ml-10,000 ml	234	20 (8.55)
10,000 ml-30,000 ml	10	10 (100.00)
30,000 ml-319000 ml	8	8 (100.00)
Total	252	38 (15.08)

Though, in the sex related distribution of microsporidium, among the 46 HIV/AIDS infected individuals, 30.43% males had viral load value/ml between 513 ml-92,300 ml and 11.65% females (926 ml-319,000 ml) most suppressed with the illness possibly that, females are most vulnerable due to their physiology and co-relate the ages between 51-60 without microsporidium had their viral load within the range of 20 ml-23 ml, this can be seen clearly that the infected patients had less viral load unlike those with microsporidium accelerate the illness. Notwithstanding, artisans show more to have habour more of the virus of 2590-319000 value/ml. Though, *Microsporidia* are important disease organisms that are considered opportunistic and this has led in recent times the improvement on the diagnostic tools to combat the organism as it show's further momentum among HIV/AIDS infected patients (Tables 5-7).

Table 5. Sex related distribution of microsporidium and viral load among HIV/AIDS infected patients.

Gender	No of HIV/AIDS patients	No positive with microsporidum spp (%)	Viral load range
Male	46	14 (30.43)	513 ml-92,300 ml
Female	206	24 (11.65)	926 ml-319000 ml
Total	252	38 (15.08)	

Table 6. Age related distribution of *microsporidium* and viral load among HIV/AIDS infected patients.

Age group	No of HIV/AIDS patients examined	No positive with microsporidum spp (%)	Viral load range
11-20	16	2 (12.50)	520 ml-56,600 ml
21-30	26	8 (30.77)	513 ml-92,300 ml
31-40	84	10 (11.90)	520 ml-56,600 ml
41-50	80	16 (20.00)	56,300 ml-58,400 ml
51-60	48	0 (0.00)	20 ml-23 ml
61-70	4	2 (50.00)	926 ml-319000 ml
Total	252	38 (15.08)	

Table 7. Occupational distribution of microsporidium and viral load among HIV/AIDS infected patients.

Occupation	No of HIV/AIDS patients	No positive with microsporidum spp (%)	Viral load range
Farmers	24	6 (25.00)	513 ml-92,300 ml
Students	91	14 (15.38)	20 ml-56,600 ml
Public/civil Servants	130	16 (12.31)	15 ml-1710 ml
Unemployed	4	1 (25.00)	1890 ml-58,4000 ml
Artisans	3	1 (33.33)	2590 ml-319000 ml
Total	252	38 (15.08)	

DISCUSSION

This present study showed the overall prevalence of *Microsporidia* among HIV/AIDS positive patients attending Federal Medical Centre, Keffi (FMC), and the general prevalence rate was 15.0% and agree with the previous case study carried out among indigenous groups in Malaysia where the prevalence rate of microsporidium ranged from 15.0% to 21.2% (Norhayati M, et al., 2007; Lono A, et al., 2010; Anuar TS, et al., 2013). The present study also showed lower prevalence rate (15.0%) compare to the study conducted in Ilorin, Nigeria with 42.4% (Nyamngee A, et al., 2013). This low prevalence could be related to location in which the study was conducted. This study has demonstrated the effectiveness of anti-retroviral therapy which is associated with the restoration of immune response accompanying the resolution of opportunistic infection including *Microsporidia* (Maggi P, et al., 2000).

Age and sex related distribution of microsporidium among HIV/AIDS patients

The intensity of spores in this present study was high amongst the old age group 61-70 years with 50.0% and those with very high viral load of 10,000 ml and above, suggesting a relationship with the level of viral load in HIV/AIDS infected patients been similar with the study carried out in Ilorin, Nigeria by Nyamngee et al., who reported high prevalence rate among the old aged group 52-61 years with 77.3%. Samie et al., reported high prevalence rate of *Microsporidium* among aged 1-10 years with 52.6% in contrast with the present study (Samie A, et al., 2021).

Marital status and sex related distribution of *Microsporidia* among HIV/AIDS Infected patients

Microsporidia was common among the males with 30.43% compared to the female with 11.65%. Those widower and singles had prevalence rate of *Microsporidia* to about 50.0% and 33.33% compared to 18.18% among the married, there was however no significant difference (X^2 cal=6.10<X^2 tab=11.07; df=5; p>0.05).

The present study is in agreement with the study carried out by Amira et al., with male had 35.50% and female 24.40% (Amira H, et al., 2018).

The present study disagreed with the earlier studies carried out by samie et al., who reported high prevalence rate (34.1) in the females compared to 26.5% among HIV/AIDS patients that indicated as male (Ghosh K, et al., 2009).

Occupation and microsporidium related HIV/AIDS infection

The occupation of the HIV/AIDS infected patients significantly affected the prevalence of *Microsporidial* infection with artisans having infected with microsporidium (33.33%) (Graczyk TK, et al., 2007). Artisans were more likely to eat food and drink water from questionable sources as they carry out their work activities. This may be the reason for the high rate of prevalence in this particular group (Weiss LM, 2020). This agrees with the findings of who observed similar risk work factor among HIV/AIDS individuals. However, contrary to the findings in North-central, Nigeria (Leelayoova S, et al., 2006).

CONCLUSION

In conclusion, this study shows that *Microsporidia* are important pathogens capable of causing opportunistic infections in severely immunodeficiency HIV infected patients. The prevalence of *Microsporidia* was high and was associated with high viral load in the present study.

The improvement in diagnostic methods and greater awareness has resulted in *Microsporidia* infections being increasingly recognized in humans. The presence of *Microsporidia* in water sources and in pets and food producing animals, along with epidemiological risk factors that have associated exposure to water and eating undercooked meat with microsporidiosis in HIV infected individuals have further raised the concern that *Microsporidia* infections may be food and water borne parasitic zoonosis. Additional epidemiological studies focusing on risk factors associated with microsporidiosis will define more clearly the sources of *Microsporidia* in the environment that pose a risk for transmission so that better preventive strategies can be implemented.

RECOMMENDATIONS

- I recommend that routine laboratory screening need to be performed for *Microsporidia* in the hospital setting and those positive results be made notifiable.
- Continued studies also are needed to identify with better accuracy the presence of viable and infectious *Microsporidia* that may pose a risk for transmission from various environmental sources.
- Methods to remove or inactivate *Microsporidia* in water sources and food still need to be develop, and more effective, less toxic drugs are needed for effectively treating microsporidiosis in human and animals.

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