Open Access

Contemporary Research Analysis Journal

e-ISSN: 3050-5909
p-ISSN: 3050-5895

Volume 02 Issue 04 April 2025

CrossRef DOI: 10.55677/CRAJ/03-2025-Vol02I04

Page no: 192-198

Prevalence of Microsporidiasis and Associated Complications among HIV-Positive Patients in North-Central Nigeria

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ABSTRACT

Introduction: A study was carried out among HIV-infected patients at the University of Ilorin Teaching Hospital, Nigeria, between January 2023 and February 2024 to determine the prevalence of microsporidiasis among HIV-infected individuals.

Methods: Seven hundred and fifty (750) stool samples were collected from HIV-infected patients and 375 samples from their non-infected counterparts (matched for age, sex and socio-economic variables). Chromoptrope 2R staining and Ficoll-Hipaque techniques were used to isolate pure microsporidia spores from the stool samples.

Results: The prevalence of microsporidia isolates in the stool samples of 750 HIV-infected patients (42.4%) was significantly higher than among the HIV-non-infected subjects (19.2%) ($P<0.05$). The highest prevalence (77.3%) of microsporidia spores was recorded among the HIV-infected patients in the age group of 52–61 years while the least prevalence (26.4%) was from the age group of 2-11years. Whereas the prevalence of microsporidiasis was higher in females (36.1%) than in males (33.1%), the difference was however not statistically significant ($P>0.05$). There were 13 observed complications of microsporidiasis among the HIV-infected patients and the prevalences of these complications were significant $P<0.05$.

Conclusion: Microsporidiasis is prevalent among immuno-compromised (HIV/AIDS) patients with varying complications. Therefore, it is desirable that a drug of choice for the treatment of microsporidiasis should be developed. Health education campaigns to promote awareness, prevention and control of microsporidiasis should be mounted and routine laboratory search and identification of microsporidia in hospitals should be made mandatory and reportable.

KEY WORDS: Prevalence, Microsporidiasis, Complications, HIV-Positive, Nigeria

INTRODUCTION

The viability of the silk industry was threatening in the early 18th century, because of a mysterious disease of silk worms. The causative agent was first named microsporidia (*Nosema bombycis*) in 1957 in a silkworm and was classified as Schizomycetes which at that time, included yeast and bacteria (Corliss and Levine 1963). Microsporidia are a diverse group of obligate intracellular eukaryotic parasite and today, approximately 1300 species in 160 genera, have been described, but this certainly represents a tiny fraction of the real diversity because most potential hosts have been poorly studied (Karimi et al 2020). Nearly all microsporidia are known to infect animals and some are responsible for a number of human diseases, predominantly associated with immuno-suppression (Wichro et al 2005). They also infect several commercially important animal species like bees, silkworms, and various domesticated animals. As earlier reported, these intracellular spore-forming protozoan that are increasingly being recognized as pathogens in humans, are ubiquitous and can infect a wide range of vertebrate and invertebrate hosts, including insects, birds, fish and mammals (Al-Brhami et al 2022). According to Javanmard *et al.*, 2018, the spores which are the infective stage vary in size but those found in humans are typically oval and 1-4µm in diameter. They are highly resistant to degradation and can survive in the environment for up to 5 months. Out of the over 140 genera and 1200 species of microsporidia infecting vertebrates and invertebrates, 15 species have been reported in humans. Within these 15 species, there are three most common ones- *Enterocytozoan bieneusi, Encephatozoan intestinalis or Septata intestinalis and Encophalitozoan cuniculi* foundly found in humans. Since the advent of HIV/AIDS as a major cause of immuno-suppression in man, the study on opportunistic organisms most of which are parasitic, has become broadened (Ismail et al 2020). These opportunistic parasites have been recognized as a major agent of chronic Diarrhoea in severely immuno-compromised patients.

In developed countries, the advent of highly active antiretroviral therapy (HAART) has been reported to reduce greatly, the prevalence of opportunistic parasitic infection in HIV-AIDS patients however, the Diarrhoea and wasting conditions in these patients still remain relevant (Halanova et al 2019). The situation is worse in developing countries where the rapid expansion of AIDS associated with limited access to HAART, compounded by ignorant and poverty are responsible for the increase in the incidences of opportunistic infections and severe Diarrhoea that often results in high morbidity and mortality profile in these immuno-suppressed group of patients. Recently, microsporidia has been identified to be responsible for this Diarrhoea and wasting in these immuno-compromised patients. It has also been reported to cause traveller's Diarrhoea even with people who have no underlying immune suppression problems (Kayaet al 2021).

Justification:
Based on the position, status, condition, circumstances, and paucity of information enumerated above, it is anticipated that the knowledge and baseline data which hitherto has remained obscured about this intracellular opportunistic protozoan, will be elucidated in this study. The knowledge thus gained will be useful for more comprehensive studies, for advocacy, for diagnosis, for prevention, and for control of microsporidiasis.

Objectives of the Study:
This study seeks to determine the prevalence and of microsporidiasis among HIV-infected patients and among their HIV-non-infected counterpart and to determine other associated other disease conditions.

METHODS

Study Design: A cross sectional study was designed to determine the prevalence of microsporidia species from human stool specimens of immuno-compromised (HIV-infected) patients and immuno-compromised (non-infected) individuals as control.

Setting: This work was carried out at the University of Ilorin Teaching Hospital (U.I.T.H), a tertiary and referral hospital located at longitude 8^0 30N and latitude 4^0 30E in Ilorin, Kwara State, Nigeria.

Preliminary Sampling: All the subjects (HIV-Positive and HIV-negative) in this study were screened for HIV-sero-status as a prerequisite for stool collection. This prerequisite distinguishes the HIV-infected patients from the HIV-non-infected control. Three screening methods, Antec HIV test, Determine test and Uni-Gold test were concurrently used to test and confirm HIV-sero-status. Questionnaires were administered on each subject whose stool samples would be collected. They provided information such as; name, sex, age, address, tribe State local government area, profession/occupation etc. A tag number was given on the questionnaire to correspond with the specimen bottle so as to ensure that each specimen corresponds with the given information.

Stool Specimen Collection: Stool specimens were obtained from both the HIV/AIDS patients and the HIV-non-infected control in sterile universal bottles. Sterile universal bottles were used for this study for collection of stool samples for two main reasons; to avoid contamination which would have been acquired environmentally that could affect isolating microsporidia spores in its pure form and to conform with the internationally accepted standard on stool collection in the contemporary world.

The Modified Trichrome Stain (Chromotrope2R) Staining Method: The Trichrome staining method was modified by the CDC Atlanta Georgia, USA, for the identification of microsporidia spores from human stool samples. method to differentiate microsporidia spores from background Faecal elements. It is specific and in most recent time, the most widely used and acceptable method for identification of microsporidia spores from stool samples worldwide (CDC 2010).
Ficoll-hypaque technique: This technique was used in this study to isolate pure microsporidia spores from the stool specimen. It is a sterile, ready to use method of density gradient mechanism for purification from human fluids, substances according to their densities using simple centrifugation procedures. Ficoll-hypaque is an aqueous solution of density 1.077±0.0019/ml, containing 5.7g Ficoll 400 and 9g sodium diatrizoate with 0.0231g calcium disodium ethylenediamintetra-a-cetic acid in every 100ml. It was acquired from Sigma chemicals co. (ca + No SL – 2).

Quality Control: As a quality control measure, a control slide of microsporidia spores in a 10% formalin preserved specimen from the CDC Atlanta, USA (No. UN 3373) [25] was viewed alongside with the prepared slides. Spore walls of microsporidia stain a pinkish-red colour and measured about 3 µm were confirmed from both self prepared and the control slides. All solutions subsequent to chromotrope stain were changed after every 10 slides to obtain proper rinsing and dehydration.

Determination of Sample Size: This sample size was predicted on the prevalence (39-97%) rate of chronic Diarrhoea among the HIV-infected patients as was determined using Fisher's formula (Araoye, 2004). An overall sample size of one thousand one hundred and twenty five (1125) humans were examined for this study. This composed of seven hundred and fifty (750) HIV-infected patients and three hundred and seventy five (375) HIV-non-infected control, matched for age, sex, and other socio-economic variables.

Data Compilation and Analysis: In the present study, the analysis of data generated was done using the statistical packages for social sciences (SPSS version 23.00). Prevalence of infection was given in percentages in line with the variables. Chi-square, student t-test and 95% Confidence interval analysis were the statistical used to determine significance. Conclusions were drawn for significant difference at 95% (p-value at 0.05).

Ethical clearance: Ethical clearance was sought for and obtained from the Ethical Review Committee of the University of Ilorin Teaching Hospital with Reference number: UITH/021/ERC2023. Relevant cooperation and assistance of various heads of clinical departments was mobilized for and granted.

Informed consent sheet: The purpose of what the study is all about was given to all subjects and participation was voluntary, the confidentiality of whatever information given for the purpose of this study was guaranteed.

RESULTS

Descriptive data:

Prevalence of Microsporidiasis among HIV-infected and HIV-non- infected Patients : At the conclusion of this study to establish the prevalence of microsporidia species among HIV Positive patients at the University of Ilorin Teaching Hospital, Ilorin, Kwara State, Nigeria, a total of 1125 patients were examined. Seven hundred and fifty (66.7%) were HIV-infected patients while 375 (33.3%) were HIV-non-infected patients. The prevalence of microsporidia infection among the 750 HIV-infected patients was 42.4%. The corresponding prevalence among the HIV-non-infected patients was 19.2%. This difference in the prevalence of microsporidia infection among HIV-infected patients and their HIV-non-infected counterparts was statistically significant ($p<0.05$) (Table 1).

Table 1: Prevalence of Microsporidiasis among HIV-infected and HIV-non- infected Patients (N=1125).

Infection Status	**No. Examined**	**No. (%) + ve***
HIV-infected patients (n)	750(66.7)	318 (42.4)
HIV-non-infected patients (n1)	375(33.3)	72 (19.2)
Total	**1125(100)**	**390 (34.7)**

P<0.05 * = Microsporidiasis.

Prevalence of Microsporidiasis by Gender: Table 2 shows that, the prevalence of microsporidiasis among HIV-infected males was 39.6% and it was 44.9% among their female counterparts. The difference in the prevalence of micrsoporidiasis among HIV-infected males and HIV-infected females was not statistically significant P>0.05. Microsporidiasis prevalence among HIV-non-infected males was 19.9% and among HIV-non-infected females was 18.6%. The difference was not statistically significant P>0.05. The total prevalence (both HIV- infected and non-infected patients) among males was 33.1% and among females was 36.1%. The difference however was not statistically significant P>0.05).

Table 2: Prevalence of Microsporidiasis among HIV-infected and HIV-non-infected Patients by Gender (N = 1125)

Infection status	**Male**		**Female**		**Total**
	No. Examined	**No. (%) +ve**	**No. Examined**	**No. (%) +ve**	**No. (%) +ve**
HIV-infected patients (n = 750)	356	141 (39.6)	394	177 (44.9)	218 (42.4)
HIV-non-infected patients (n1=375)	176	35 (19.9)	199	37 (18.6)	72 (19.2)
Total (N=1125)	**532**	**176 (33.1)**	**593**	**214 (36.1)**	**390 (34.7)**

P>0.05

N-total number of subjects in the study n-total number of HIV-Seropisitive patience, n1-Total number of other patients used as control in the study.

Combined Prevalence of Microsporidiasis by Age and HIV Status: As shown in table 3, the highest prevalence (77.3%) of microsporidiasis among the HIV-infected patients was in the oldest age group of 52-61 years. Also, the highest prevalence (44.4%) of micrsoporidiasis among HIV-non-infected patients was in the same oldest group of 52-61 years. The least infection rate of microsporidiasis of 26.4% and 0.0% were recorded in the age group of 2-11 years for both the HIV-infected and the HIV-non-infected patients respectively.

The prevalence of microsporidiasis among the HIV-infected patients increased with age from the age group of 32-41years (58.6%) to 52-61 years (77.3%), but, there was no consistency in the prevalence of microsporidiasis among their non-infected counterparts in the same age groups.

Table 3: Combined Prevalence of Microsporidiasis by Age among the HIV-infected and HIV-non-infected Patients.

Age group (years)	**HIV-infected Patients**		**HIV-non-infected**		Total	
	No. Examined	**No.(%)+ ve**	**No. Examined**	**No.(%)+ve**	**No. Examined**	No.(%) +ve
≤ 1	11	3(27.3)	6	2(33.3)	17	5(29.4)
2-11	53	14(26.4)	28	0(0.0)	81	14(17.3)
12-21	175	56(32.0)	108	14(13.0)	283	70(24.7)
22-31	238	76(31.9)	113	20(17.7)	351	96(27.4)
32-41	152	89(58.6)	64	27(42.2)	216	116(53.7)
42-51	99	63(63.4)	47	5(10.6)	146	68(46.6)
52-61	22	17(77.3)	9	4(44.4)	31	21(67.7)
Total	750	318(42.4)	375	72(19.2)	1125	390(34.7)

Prevalence of Microsporidiasis in Relation to Different Disease Conditions: The highest prevalence of microsporidiasis among the 13 listed disease conditions was from cough + Diarrhoea + body rashes (100%), Diarrhoea + body rashes (100%) and body rashes (91.7%) while the least prevalence of microsporidiasis was found among HIV- infected patients with tumor cell (cancer) 44.4%. The prevalences of microsporidiasis among all the disease conditions were statistically significant (Table 4).

Table 4: Prevalence of Microsporidiasis by Different Disease Conditions Associated with the 750 HIV-infected Patients.

S/No.	**Disease Conditions**	**No. examined in each case (n)**	**No.(%) +ve for microsporidia**	**CD4+ Counts for the microsporidia + ve samples**	**Mean ±SD of CD4+ for microsporidia +ve samples.**	P-value by 95% confidence interval
1	HIV Seropositive (HSP)	750	318(42.4)	344	219±106	0.007
2	Pregnancy + HSP	28	21(75.0)	234	127±76	0.002
3	Cough (T.B) + HSP	216	108(50.0)	216	142±84	0.006
4	Tumor Cell (Cancer) +HSP	9	4(44.4)	132	96±50	0.007
5	Diarrhoea + HSP	482	304(63.0)	120	87±48	0.004

6	Body Rashes + HSP	216	198(91.7)	103	74±36	0.001
7	Fever + HSP	272	192(70.7)	122	88±41	0.002
8	General Body weakness + HSP	192	93(48.4)	243	92±36	0.006
9	Diarrhoea, Cough +HSP	296	228(77.0)	181	86±42	0.002
10	Fever, Diarrhoea +HSP	396	209(52.8)	172	76±28	0.003
11	Diarrhoea, Body rashes + HSP	298	298(100.0)	114	69±23	0.000
12	Body rashes, cough+ HSP	273	191(70.0)	111	62±21	0.005
13	Cough(TB),Diarrhoea, Body rashes + HSP	302	302(100.0)	97	11±6	0.000

P<0.05. HSP-HIV-Sero-Positive, TB-Tuberculosis, SD- Standard Deviation, +ve-Positive.

DISCUSSION

The overall prevalence of microsporidiasis recorded in this study (42.4%) is relatively high for an infection which is hardly known or considered significant or important among its hosts. The high prevalence among the HIV-infected patients strongly suggests that microsporidiasis is relatively common among the HIV-infected patients at the University of Ilorin Teaching Hospital, Ilorin, North-central Nigeria. The study confirms the speculations that, if properly investigated, microsporidiasis prevalence among the immuno-compromised groups could be alarmingly high (Bojko et al 2022).

The prevalence of microsporidiasis found among this subject poses a concern particularly when it was discovered further from this study that the infection cuts across all ages, sex, educational status, religion and other socio-demographic variables. Like in the HIV-infected patients, the young and the old were also seen to habour microsporidia among the HIV-non-infected control, conforming the wide spread and epidemiological relevance of this organism. The high prevalence rate of microsporidia infection among the HIV-infected infants supports the argument that transplacental transmission from mother to fetus as it is in the case of HIV-sero-status of mothers is possible in microsporidiasis (Vecková et al 2021). Unlike other parasitic infections which are either age or occupationally related, microsporidiasis shows its distribution in infants and the aged to be relatively high. However, the ubiquitous and highly cosmopolitan nature of microsporidia species as documented by Bryan *et al.*,(1997) is a possibility of acquiring infection even by infants. This ubiquitousness of the parasite has been supported by findings from this study where all age groups, all sexes, civil servants or farmers irrespective of social class and all ethnic groups were significantly infected (Diao et al 2022).

Furthermore, the sex related prevalence where 33.1% and 36.0% were reported for males and females respectively confirms the assumptions by Wang *et al* 2018 that this infection has no gender bias and has high cosmopolitan potentials. This highest prevalence as recorded among the aged HIV-infected patients explains that, microsporidiasis when acquired and not treated is capable of successful existence as one ages, particularly when conditions that favour the rapid production of the spore are favourable. And except when treated with, yet to be identified drug of choice, microsporidia has not been responding effectively to other drugs like HARRT which could assist in eliminating other opportunistic infections in the immuno-compromised patients. The infection as seen, remain relevant, particularly among the aged immuno-compromised patients, thus confirming what Ayinmode *et al* (2018) that, intestinal microsporidiasis in human immunodeficiency virus-infected patients with Diarrhoea in Germany is alarming. The argument appears to be unnecessary, as findings from this study which revealed that microsporidia was prevalent despite HARRT treatment, contradicts the earlier assumptions and confirms the latter. It is expedient to note that, the difference in the biology of microsporidia, its mode of infection and the ability of the spores to thrive well on any living cell are facts that support the argument that microsporidia persist despite HARRT treatment.

As reported by Bryan *et al* (1997), that microsporidia is prevalent in immuno-competent individuals and that the infection is responsible for the travellers Diarrhoea in the immuno-competent hosts, findings from this study which reveal a prevalence rate of 19.2% in the HIV-non-infected strongly supports their argument. This prevalent rate is relevant, particularly of the fact that, similar clinical features like Diarrhoea which are common to the HIV-infected patients were also seen in the HIV-non-infected control. Meanwhile, the Diarrhoea which was chronic in the HIV-infected patients, was intermittent and mild in the HIV-non-infected individuals. Like Wichro *et al.* (2005) who reported an unexpectedly very high prevalence (67.5%) of microsporidiasis in 126 immuno-competent individuals, otherwise healthy people, findings from this study revealing 19.2% prevalence and their surprising revelation show cause to worry about the ubiquitous nature of microsporidia species. Although Valenčáková & Danišová (2019), concluded that, there was no clinical manifestations in these individuals, diligent, meticulous and careful study as revealed

by this research work presents mild Diarrhoea that is characteristic of microsporidiasis among the 19.2% prevalence recorded. Earlier Wichro *et al* (2005) concluded that travellers Diarrhoea as a result of microsporidiasis remain high in healthy individuals, conforming with findings from this study. Wichro *et al* (2005), had earlier alerted the world in their report of an emerging infectious disease, which according to him will posse a great public health and socio-economic challenges on humans worldwide. He intimated researchers of the urgent need for a thorough search for the etiological agent of the Diarrhoea situation among the immuno-compromised group. As findings from this study reveals, they also quarried microsporidiasis to be responsible for this condition among the immuno-compromised (HIV-infected) patients.

Among the HIV-infected patients also, the 100% prevalences of microsporidiasis reported in patients with combined Diarrhoea + body rashes and Diarrhoea + cough + body rashes as disease conditions in HIV-infection presents how important microsporidia infection can complicate situations in these patients. This shows that, although not conclusive, HIV/AIDS patients presenting themselves with these complications could almost be clinically diagnosed to have Microsporidia infection. In close association to these findings, Han & Pan (2021) had already concluded that Diarrhoea complication in HIV-infections is of clinical essence in diagnosing opportunistic infections. All these complications as seen in this study present a high morbidity and consequent mortality among the HIV/AIDS patients. Earlier as reported by Ghoshal *et ai* (2021) where a 37% mortality rate in HIV/AIDS patients as a result of opportunistic infection was documented, presenting these complications, from this study then confirms that microsporidiasis is highly implicated in the mortality rate among HIV/AIDS patients. Unlike other opportunistic infections, microsporidiasis as observed, is accompanied by chronic Diarrhoea among the HIV-infected patients, causing high morbidity and mortality rates in this group of patients. Among the 318 (42.4%) microsporidia – positive HIV-infected patients, it was observed that 304 (95.6%) persistently reported with chronic Diarrhoea. Like in malaria where the onset of fever produces the first noticeable sign days after the bite of an infected mosquito, microsporidia also has shown close resemblance, where it was detected in some HIV-infected patients who had no Diarrhoea complications. The latent phase of the high resistant profile of the parasite could not be left out as a possibility of long infection before clinical signs. This was confirmed in the study where low intensity was reported in group of HIV-patients on HARRT but complications like wasting, Diarrhoea, body rashes and cough still remain high in them and where Diarrhoea was seen to re-surface with high magnitude, days after faulting any HARRT regimen. This Diarrhoea in HIV-infection was seen irrespective of whether these patients were on HAART treatment or not, hence creating worry on the efficacy of HARRT treatment on microsporidiasis. The presence of micrsoporidia among the HIV-infected patients on HARRT treatment suggest that; microsporidia is highly implicated in the morbidity rate caused by the chronic Diarrhoea in the HIV-infected patients and that, the HAART treatment/drugs may not be effective against microsporidia as supposed in other opportunistic infections. Furthermore, this study reveals that, microsporidia will remain a parasite of interest for further studies in the morbidity and mortality rates among the immuno-compromised patients worldwide. While much more is expected to be established about microsporidia, findings from this initiative are encouraging and enough to substantiate that, epidemiological significance of this parasite and knowledge about the parasite are relevant to man.

CONCLUSION

This study concludes that the prevalence of microsporidiasis (an opportunistic infection) is common among the immuno-compromised (HIV/AIDS) patients, thus posing a serious threat to complicate morbidity and mortality in areas where HIV/AIDS is common. And that Microsporidiasis affects all age groups, all sexes thus, significant diarrhoea, cough, body rashes and potentially death were the observed complications of microsporidia infection in the immuno-suppressed (HIV/AIDS) patients. We recommend that a drug of choice for the treatment of microsporidiasis should be developed. Health education campaigns to promote awareness, prevention and control of microsporidiasis should be mounted and routine laboratory search and identification of microsporidia in hospitals should be made mandatory and reportable.

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