

Prevalence of pulmonary histoplasmosis infection among Human Immunodeficiency Virus/Tuberculosis patients attending infectious diseases hospital, Kano, northwestern Nigeria

Sumayya Shuaibu Ibrahim,¹
Isah Abubakar Aliyu,²
Taysir Ramadan,¹ Umar Usman,³
Yusuf Mohammad Sabo¹, Firdausi
Abubakar,⁴ Maryam Kabir Umar,⁵
Mustapha Ahmed Yusuf¹

¹Department of Medical Microbiology and Parasitology, Faculty of Clinical Sciences, Bayero University, Kano;

²Department of Medical Laboratory Science, College of Health Sciences, Bayero University, Kano;

³Department of Biology, School of Science- Jigawa State College of Education, Gumel;

⁴Department of Family Medicine, Aminu Kano Teaching Hospital, Kano;

⁵Department of Microbiology, Federal University, Dutsin-ma, Katsina, Nigeria

Abstract

Histoplasmosis is a disease with a global distribution and hyperendemic zones. The severity of the sickness is determined by the number of conidia inhaled and the host's cellular immune system's performance. This study aimed to determine the prevalence of pulmonary histoplasmosis infection among Human Immunodeficiency Virus (HIV) / Tuberculosis (TB) patients attending an infectious diseases hospital in Kano, Nigeria. The study is a cross-sectional hospital study, in which 203 interviewer-administered questionnaires were distributed, sputum samples were collected, and fungal culture was carried out. Identification and antifungal susceptibility for the test isolates were done using microscopic examination and agar disc diffusion. Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 22.0. The prevalence of histoplasmosis was 5.4%. Of the 67 (33.0%) samples collected from TB patients, only 5 (2.5%) were positive for *Histoplasma capsulatum*, and of the 75 (37.0%) samples collected from HIV patients, only 4 (2.0%) were positive. The findings highlight the need for developing better diagnostic tools that will promptly make the diagnosis of the infection, espe-

cially in developing countries. Histoplasmosis can present with symptoms similar to other respiratory diseases, which may lead to delays in treatment, and increase the financial burden of managing the infection on the patients.

Introduction

Histoplasmosis is a disease with a global distribution and hyperendemic zones. The severity of the disease is determined by the number of conidia inhaled and the host's cellular immune system's performance.¹ The most common symptom of histoplasmosis is pulmonary infection, which can range from mild pneumonitis to severe acute respiratory distress syndrome.² *Histoplasma capsulatum* is commonly disseminated within macrophages and becomes symptomatic in patients with cellular immune deficiencies.³ Acute, severe, life-threatening sepsis and chronic, slowly advancing illness are both examples of disseminated infection.⁴

The World Health Organization (WHO) estimated the incidence of tuberculosis in Nigeria to be 322 per 100,000 people, with only 15% of the entire burden of the disease being recorded with Genexpert and/or acid-fast alcohol smear.⁵ Histoplasmosis is an important differential diagnosis in Human Immunodeficiency Virus (HIV) infected patients with Tuberculosis (TB).⁶ In Nigeria, data on histoplasmin skin sensitivity screening dates back over three decades and was collected before the HIV crisis.⁷ Given that disseminated histoplasmosis is an AIDS-defining condition with a high mortality rate, and there is a paucity of data, it is vital to investigate the exposure in this group of patients.⁸

Materials and Methods

Study area

The study was carried out at the Infectious Diseases Hospital (IDH) in Kano, Nigeria. IDH is a specialized service hospital located at Sabon Gari, Fagge Local Government area of Kano State. It is located at the latitude of 12°5'29.67N and longitude of 8°29'46.81E or 12.091575 and 8.496336, respectively.⁹

Study design

This study is a cross-sectional hospital-based study.

Correspondence: Mustapha Ahmed Yusuf, Department of Medical Microbiology and Parasitology, Faculty of Clinical Sciences, Bayero University, Kano, Nigeria. Tel. +2348036272989. E-mail: mayusuf1315@gmail.com

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Availability of data and materials: all data generated or analyzed during this study are included in this published article.

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Data collection

Data was collected using a preform structured questionnaire, physical examination, and clinical diagnosis were also noted from the patients' records.

Determination of sample size

The sample size for the study was determined from the sample size.

$$n = \frac{(Z_{1-\alpha})^2(P)(1-P)}{d^2}$$

where;

n = minimum sample size

Z_{1-α} = value of standard normal deviation, which at 95% confidence interval was found to be 1.96.

P = the best estimate of prevalence obtained from the literature review (14.0%) based on a previous study by Muhammad and Muhammad, 2019, in Kano State.

d = difference between the true population rate and sample that can be tolerated; this is the absolute precision (in percentage) on either side of the population.

$$n = \frac{(1.96)^2(0.14)(1-0.095)}{(0.05)^2}$$

Therefore, 193 with 5% attrition (10) giving a minimum sample size of 203 distributed according to the estimated daily turnover (75 (37%), 67 (33%) and 61 (30%) was used.

Study population

A total number of 203 patients with HIV, TB, and HIV/TB co-infection were used.

Sample collection

The patients were instructed on how to collect their sputum samples aseptically. Five milliliters (about 1 teaspoon) of early morning sputum were collected aseptically using labeled, sterile, wide-mouthed, screw-capped plastic sputum containers.

Sample processing

Microscopic examination

The specimen was examined microscopically using 10x and 40x objectives with the condenser iris diaphragm closed sufficiently to give a good contrast.

Potassium Hydroxide (KOH) preparation

Drops of KOH solution were put on a slide. The sputum (small piece) was transferred to a drop of KOH, and covered with glass. The slides were placed in a Petri dish or other container with a lid, together with a damp piece of filter paper or cotton wool, to prevent the preparation from drying out.

Lactophenol Cotton Blue (LCB) mount preparation

A large drop of LCB was put on a clean, grease-free glass slide using a Pasteur pipette. A small quantity of the sputum was transferred to the drop. The culture was teased with the teasing needles as to get a

uniform spread. The cover slip was put on to avoid entrapment of air bubbles.

Culture

Drops from each sputum sample were inoculated on Sabouroud Dextrose Agar medium (SDA) amended with chloramphenicol, for primary isolation of *Histoplasma capsulatum*.¹⁰ The plate was incubated at 37 °C and examined after 24-48 hours.

Inoculation and incubation of samples

Samples were inoculated on SDA and incubated at 25°C in Bio-Oxygen Demand (BOD) for four weeks. SDA bottles were examined for growth once in two days during the 1st week and twice a week thereafter for up to 4 weeks. SDA media with growth were examined by Gram staining method and microscopy.

Gram staining

The cultured colony was subjected to gram staining for morphological identification of test isolates.

Procedure

A smear of the colony was prepared on a glass slide and allowed to air dry, and then the fixed material was passed through the flame of a Bunsen burner. The smear was placed on a staining rack and the surface was overlaid with crystal violet solution for one minute, and then washed thoroughly with distilled water. Then the smear was also overlaid with grams iodine for one minute and washed again with water. Then it was flooded with few drops of acetone alcohol decolorizer until no violet color was seen which usually took ten seconds or less and it was then washed with running water. The smear was placed on a staining rack and overlaid the surface with Safranin counterstain for one minute, then washed with running water. The smear was placed in an upright position on the staining rack and allowed the excess water to drain off and dry the smear. Then the stained smear was examined under X100 oil immersion.¹¹

Ethical clearance

Ethical clearance was obtained from the Research and Ethics Committee of the

Table 1. Socio-demographic information and prevalence of *H. capsulatum*.

Parameter	Positive (%)	Negative (%)	pe
Age			
11-20	1 (0.5)	10 (4.9)	0.031
21-30	0 (0.0)	61 (30)	
31-40	3 (1.5)	63 (31.0)	
41-50	5 (2.5)	39 (19.2)	
51-60	2 (1.0)	11 (5.4)	
>60	0 (0)	8 (3.9)	
Total	11 (5.4)	192 (94.6)	
Gender			
Male	5 (2.5)	98 (48.3)	0.718
Female	6 (3.0)	94 (46.3)	
Total	11 (5.4)	192 (94.6)	
Educational status			
Illiterate	4 (2.0)	58 (28.6)	0.469
Primary	4 (2.0)	41 (20.2)	
Secondary	2 (1.0)	46 (22.7)	
Tertiary	1 (0.5)	47 (23.2)	
Total	11 (5.4)	192 (94.6)	
Occupation			
Civil servant	2 (1)	37 (18.2)	0.724
Farmer	4 (2.0)	53 (26.1)	
Poultry workers	1 (0.5)	21 (10.3)	
Roofers	0 (0.0)	29 (14.3)	
Business	4 (2.0)	52 (25.6)	
Total	11 (5.4)	192 (94.6)	

Table 2. Prevalence of *H. capsulatum*.

Type of infection	Positive (%)	Negative (%)	p
Human Immunodeficiency Virus (HIV)	4 (2.0)	71 (35.0)	0.633
Tuberculosis (TB)	5 (2.5)	62 (30.5)	
HIV/TB co-infection	2 (1.0)	59 (29.1)	
Total	11 (5.4)	192 (94.6)	

Kano State Ministry of Health before the commencement of the study. An informed consent was also obtained from each patient before enrolment into the study.

Results

Table 1 shows that the age group 41-50 has the highest number of positive samples, 5 (2.5%), as compared to other age groups. The table reveals that there is no significant difference within the three categories of patients, $p=0.633$. The table indicates that female participants recorded the highest positive samples as compared to the males, with 6 (3.0%) and 5 (2.5%) out of 94 (46.3%) and 98 (48.3%) patients, respectively. There is no significant difference between the gender, $p=0.718$. On the other hand, the results of the patients on the basis of their educational status reveal that those with non-formal education and primary level have the highest positive samples, with 4 (2.0%) each out of 58 (28.6%) and 41 (20.2%) patients, respectively. This is followed by secondary school, with 2 (1.0%) out of 46 (22.7%) samples collected from the patients. Tertiary level has the least positive sample of 1 (0.5%) out of 47 (23.2) samples collected. There is no significant difference, $p\text{-value}=0.469$. Similarly, the table indicates the results of the participant on the basis of their occupations. Thus, farmers and business participants recorded the highest positive samples, with 4 (2.0%) each out of 57 (28.1%) and 56 (29.6%), respectively. This is followed by poultry workers, 1 (0.5%) out of 22 (10.8%).

Table 2 shows the overall prevalence, 11 (5.4%), of histoplasmosis. The total number of samples collected from HIV/TB patients and HIV-TB co-infection patients was 203. Out of 203 samples collected, 75 and 67 were HIV and TB patients, respectively. Only 61 samples were collected from patients with HIV and TB co-infection. The table shows that out of 67 (33.0%) samples collected from TB patients, only 5 (2.5%) were positive for *Histoplasma capsulatum*, and out of 75 (37.0%) samples collected from HIV patients, only 4 (2.0%) were positive. However, the table reveals that out of 61 (30.0%) samples collected from HIV and tuberculosis co-infection, only 2 (1.0%) samples were found positive for *Histoplasma capsulatum*. TB recorded the highest positive samples of 2.5%, followed by HIV 2.0%. The table also reveals that the statistical analysis carried out showed that there was no significant difference within the three categories of patients, $p=0.633$.

The prevalence of *Histoplasma capsu-*

latum among HIV patients found in the current study was 4 (2.0%) out of 75 (37.0). However, the prevalence of *Histoplasma capsulatum* among tuberculosis patients found in the current study was 5 (2.5%) out of 67 (33%). On the other hand, the prevalence of *Histoplasma capsulatum* among HIV and tuberculosis co-infected patients found in this study was 2 (1.0%) out of 61 (30.0%). Histoplasmosis was suspected in 203 patients based on their clinical and laboratory characteristics. *Histoplasma capsulatum* among tuberculosis patients revealed the highest prevalence rate, 5 (2.5%). This is followed by HIV patients, with 4 (2.0%). However, those patients with HIV and tuberculosis co-infection recorded the lowest prevalence rate, 2 (1.0%).

Discussion

The results of this study show that histoplasmosis is a rare opportunistic infection among HIV and TB patients in the study area, with an overall prevalence of 5.4%. This is lower than the prevalence reported in other studies.^{12,13} The low prevalence of histoplasmosis in the area may be due to several factors, such as the low incidence of *Histoplasma capsulatum* in the environment, the low exposure of the population to the fungus, or the underreporting of cases due to lack of awareness or misdiagnosis.^{14,15}

The study also reveals that there is no significant difference in the prevalence of histoplasmosis among the three categories of patients: HIV, TB, and HIV-TB co-infection. This suggests that histoplasmosis is not associated with a specific immunosuppressive condition, but rather with a general impairment of the host defense mechanisms. However, it is noteworthy that TB patients had the highest proportion of positive samples (2.5%), followed by HIV patients (2.0%), and HIV-TB co-infected patients (1.0%). This may indicate that TB infection increases the risk of acquiring histoplasmosis, possibly by facilitating the inhalation or dissemination of *Histoplasma capsulatum* spores.^{16,17} Conversely, HIV-TB co-infection may have a protective effect against histoplasmosis, possibly by inducing a Th1 immune response that limits the growth of the fungus.^{18,19} However, these hypotheses need to be confirmed by further studies with larger sample sizes.

The study also shows that there is no significant difference in the prevalence of histoplasmosis according to age, gender, education level, or occupation of the patients. This implies that histoplasmosis is not influenced by demographic or socioeco-

nomical factors, but rather by environmental or biological factors. However, it is interesting to note that female patients had a slightly higher prevalence than male patients (3.0% vs 2.5%), and that patients with non-formal education and primary level had a higher prevalence than those with secondary or tertiary level (2.0% vs 1.0%). This may suggest that female patients and those with lower education levels are more exposed to *Histoplasma capsulatum* spores in their daily activities, such as farming, gardening, or animal handling.^{20,21} However, these findings should be interpreted with caution, as they may be confounded by other variables, such as access to health care, adherence to treatment, or comorbidities.

Conclusions

In conclusion, this study provides valuable information on the epidemiology and antifungal susceptibility of histoplasmosis among HIV and TB patients. The results indicate that histoplasmosis is a rare but potentially fatal infection in this population, and that it is not associated with any specific immunosuppressive condition or demographic factor. The study also demonstrates that *Histoplasma capsulatum* is sensitive to amphotericin B, itraconazole and ketoconazole, but resistant to fluconazole. These findings have important implications for the diagnosis and treatment of histoplasmosis in Nigeria and other resource-limited settings.

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