

PREVALENCE PROFILES OF CRYPTOCOCCAL ANTIGENEMIA AMONG HIV/AIDS PATIENTS ATTENDING ANTIRETROVIRAL THERAPY CLINIC AT MASAKA REGIONAL REFERRAL HOSPITAL, MASAKA DISTRICT IN UGANDA. A CROSS-SECTIONAL STUDY.

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ABSTRACT

Background

Cryptococcosis is an opportunistic infection with *Cryptococcus neoformans* a fungus that lives in the environment worldwide. The study aimed to determine the Prevalence profiles of Cryptococcal antigenemia among HIV/AIDS patients attending the Antiretroviral Therapy Clinic at Masaka Regional Referral Hospital, Masaka District in Uganda

Methodology

A cross-sectional study included HIV-positive patients receiving ART who attended the HIV/AIDS clinics at Masaka Regional Referral Hospital – ART Clinic. Blood samples were analyzed for *Cryptococcus neoformans* and *Cryptococcus meningitis* antibodies.

Results

45.54% of the respondents were females and 54.46% were males. The majority (85.0%) of participants were aged 15-44. The prevalence of cryptococcal antigenemia was 8.91% (9/101). Prevalence amongst females was 15.2% (7/46) and 3.6% (2/55) amongst males. Males accounted for 22% (2/9) and females accounted for 78% (7/9) of the positive cases identified.

Conclusion

The prevalence of cryptococcal antigenemia was higher in females compared to males among participants aged 15-44 years.

Recommendation

Regular Immune Status Monitoring by implement regular monitoring of CD4 counts to identify patients at higher risk of opportunistic infections and ensure timely intervention.

Keywords: Prevalence profiles, Cryptococcal antigenemia, Human Immunodeficiency Virus / Acquired Immune Deficiency Syndrome patients, Antiretroviral Therapy Clinic

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BACKGROUND OF THE STUDY

Cryptococcosis is an opportunistic infection with *Cryptococcus neoformans* a fungus that lives in the environment throughout the world and primarily affects people with advanced Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) which is an important cause of morbidity and mortality around the globe (Rasul CH, 2012). HIV infection is the main risk factor estimated to account for 95% of cases in middle and low-income countries and 80% of the cases in high-income countries (Geda N *et al.*, 2019).

In sub-Saharan Africa, a high diversity of cryptococcal antigenemia prevalence was noted ranging from 2% to 21% (Manga N *et al.*, 2016) with Senegal having a 9.2% prevalence of positive cryptococcal antigenemia. A study

by Derby A *et al.*, (2020) reported (a 7%) cryptococcal antigenemia proportion in Ethiopia while Thomsen *et al.*, (2018) reported that, of HIV patients included in a study in Guinea-Bissau, (10%) had a positive cryptococcal antigen test.

Studies on cryptococcal antigen screening and outcomes in Uganda have been limited mostly to central Uganda. They largely focused on ART naïve patients and those with CD4 cell counts of 100 cells/mm³ and below. To the best of my knowledge, the prevalence of Cryptococcal antigenemia among PLHIV in this community in central Uganda is unknown.

This study therefore aims at determining the prevalence of cryptococcal antigenemia among people with HIV disease (Either CD4 200cells/mm³ and below or stage 3 or 4 World Health Organization (WHO) clinical stage event at

presentation irrespective of CD4 cell count) in Masaka regional referral hospital.

METHODOLOGY

Study design

A cross-sectional study included HIV-positive patients receiving ART who attended the HIV/AIDS clinics at Masaka Regional Referral Hospital – ART Clinic. Blood samples were analyzed for *Cryptococcus neoformans* and *Cryptococcus meningitis* antibodies. The study was conducted from April to June 2024.

Study area

The study was conducted at the ART Clinic to investigate the aspects of Cryptococcal infections at Masaka Regional Referral Hospital. Located situated in Masaka City along the Masaka–Mbarara Highway approximately 132 kilometers (82 mi), by road, away from Kampala, accessible via Alex-Ssebowa Road in Katwe Town. The coordinates of Masaka Regional Referral Hospital are 0°19'46.0"S, 31°44'04.0" E (Latitude: -0.329444; Longitude:31.734444). This location was chosen due to its central accessibility and the high concentration of HIV/AIDS patients seeking treatment and care at the hospital's ART Clinic.

Study population

The study enrolled all patients aged 15 years and older who were attending the ART clinic during the study period at Masaka Regional Referral Hospital.

Sample size determination

The sample size was determined using the Kish and Leslie formula (2000) as stated below.

Kish and Leslie's formula, $n = \frac{z^2 pq}{d^2}$

Where, n = the required sample size

z = the standard deviation (1.96) at a 95% confidence interval

d = allowed error (0.05)

p = estimated prevalence of Cryptococcal antigenemia among HIV patients in Uganda (7.1%) (Ebonu E, 2021) hence p (0.071).

q = (1-p)

z = 1.96, p = 0.071, d = 0.05, q = (1-0.071)

Therefore, $n = \frac{1.96 \times 1.96 \times 0.071 \times 0.929}{0.05 \times 0.05}$

n = 101

A total of 101 patients diagnosed clinically with meningitis were included in the Study.

Sampling technique

The study employed a convenience sampling technique, selecting participants based on their availability and willingness to participate, which suited the practical constraints of the research setting.

Sampling Procedure:

The study utilized a convenient sampling approach to recruit participants from the ART clinic. HIV/AIDS patients attending the clinic were informed about the study objectives. Those who provided consent and had a CD4 count of less than 200 cells/ μ L were eligible for enrollment in the study.

Data collection method

Closed-ended questionnaires were employed to gather comprehensive qualitative and quantitative data, encompassing demographic factors, obstetric history, and socioeconomic indicators such as occupation, income levels, and educational attainment.

To ascertain prevalence, blood samples were systematically collected using either red-top or purple-top vacutainers from all participants. These samples were subsequently subjected to analysis using the Cryptococcal antigen (CrAg) lateral flow assay (LFA).

Data collection sheets were meticulously utilized to record and compile the laboratory results obtained from the analysis process.

Data collection tool(s)

Demographic information including age, place of residence, and marital status was gathered using a questionnaire featuring a mix of closed and open-ended questions. This facilitated comprehensive data collection from the respondents.

Quantitative data was obtained through the use of the CrAg LFA (Cryptococcal antigen lateral flow assay). This diagnostic method operates on the principle of a dipstick sandwich immunochromatographic assay. It is specifically designed to detect cryptococcal antigens present in either serum or whole blood samples.

The CrAg Kit employed in the study provided rapid and reliable results, aiding in the accurate assessment of Cryptococcal antigenemia among the participants.

Data collection procedure

The researchers first introduced themselves to HIV/AIDS patients, explaining the purpose of the study and obtaining consent from willing participants. Each eligible participant received a questionnaire to ensure accurate data collection. To facilitate identification, a unique number was assigned to every patient. Venous blood was then collected using aseptic techniques and deposited into appropriately labeled

red-top or purple-top vacutainers as per standard operating procedures (SOPs).

Upon collection, samples were centrifuged to separate serum (from the red top) or whole blood (from the purple top).

A process followed where one drop of specimen diluent was added to a test tube, followed by 40 microliters of patient serum or whole blood, which was thoroughly mixed. Subsequently, a CrAg strip was inserted into the test tube with the mixed sample and allowed to react for 10 minutes. After the designated time, the results were interpreted and recorded on the data collection sheet.

The findings from these procedures were instrumental in determining the prevalence of Cryptococcal antigenemia among HIV/AIDS patients in the study.

Study variables

The study's dependent variable focused on determining the prevalence of cryptococcal antigenemia among HIV/AIDS patients. The independent variable comprised individuals receiving antiretroviral therapy (ART) for HIV/AIDS.

These variables were crucial in examining the relationship between ART treatment and the occurrence of cryptococcal antigenemia among the study participants.

Piloting the study

A pilot study involving 8 participants exhibiting signs and symptoms highly suggestive of cryptococcal infection was conducted to assess the feasibility and refine the methodology for the main study.

This preliminary investigation aimed to gather initial insights into the diagnostic procedures and data collection techniques that would be employed in the subsequent larger-scale study.

Quality control

Before data collection commenced, the researcher conducted training sessions for research assistants to ensure accurate results were obtained. The research tools underwent a pre-test phase to identify and address any necessary adjustments before the main study.

The serum cryptococcal antigen (CrAg) serology test adhered strictly to the manufacturer's instructions. During testing, both negative and positive control samples were included alongside the study samples to validate the accuracy and reliability of the results.

Data analysis and presentation

Patient data was collected through responses provided in the administered questionnaires, while laboratory data was gathered from the results of conducted tests. Subsequently, the data was processed and analyzed using Microsoft Excel for thorough examination.

The findings were then compiled into figures, such as pie charts and bar graphs. These visual representations were utilized to effectively present and interpret the generated information from the study.

Ethical consideration

The research commenced with the acquisition of an introductory letter from the Research and Ethics Committee of the Faculty of Health Sciences at the University of Kisubi. This letter was then submitted to the administration of Masaka Regional Referral Hospital to obtain formal permission to conduct the study. Upon receiving a letter of acceptance from the hospital administration, data collection promptly commenced.

Throughout the study, the utmost confidentiality of patient information was assured, and participants were informed of their right to withdraw from the study at any point without consequences. These ethical considerations were strictly adhered to during the entire research process.

RESULTS

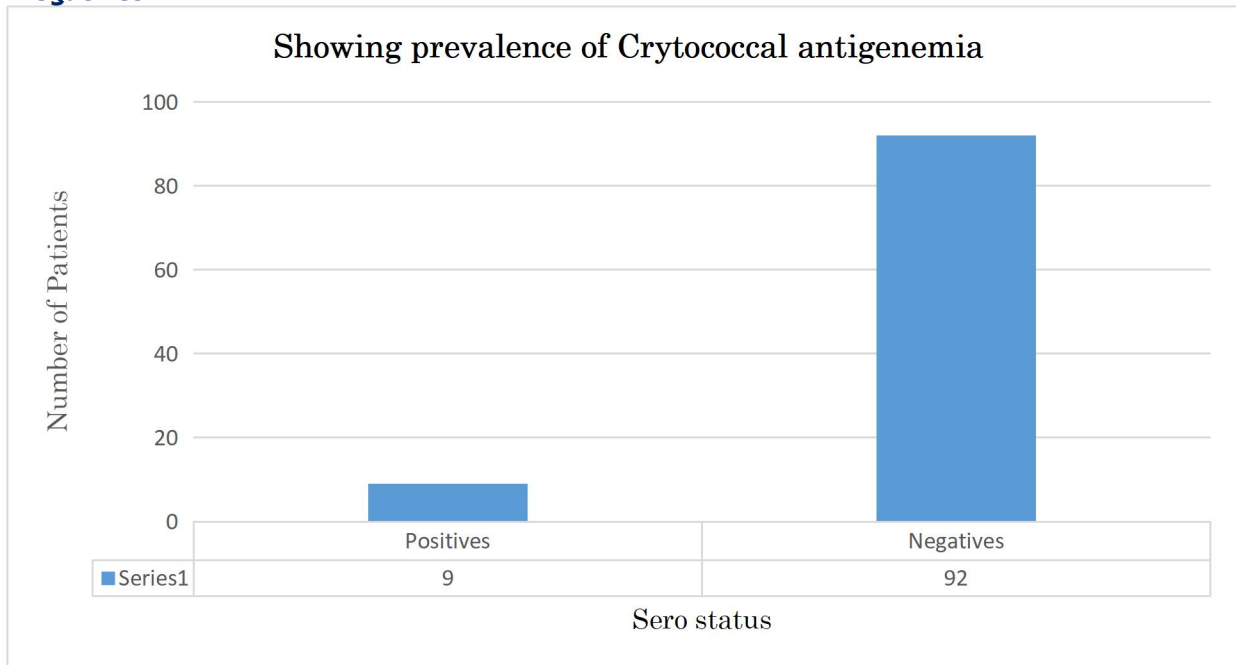
Socio-demographic characteristics

During the study conducted from April to June, 101 clients were enrolled, comprising 45.54% females and 54.46% males. The majority (85.0%) of participants were aged 15-44, with the remaining 15.2% aged 45 years and older.

Prevalence profiles of Cryptococcal antigenemia among HIV/AIDS patients attending Antiretroviral Therapy Clinic at Masaka Regional Referral Hospital, Masaka District in Uganda

The prevalence of cryptococcal antigenemia was 8.91% (9/101). Prevalence amongst females was 15.2% (7/46) and 3.6% (2/55) amongst males. Males accounted for 22% (2/9) and females accounted for 78% (7/9) of the positive cases identified.

Figure 1 illustrates the number of patients categorized into two groups: "Positives" and "Negatives."



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Figure 2 illustrating the number of patients categorized into two groups: "Females" and "Males."

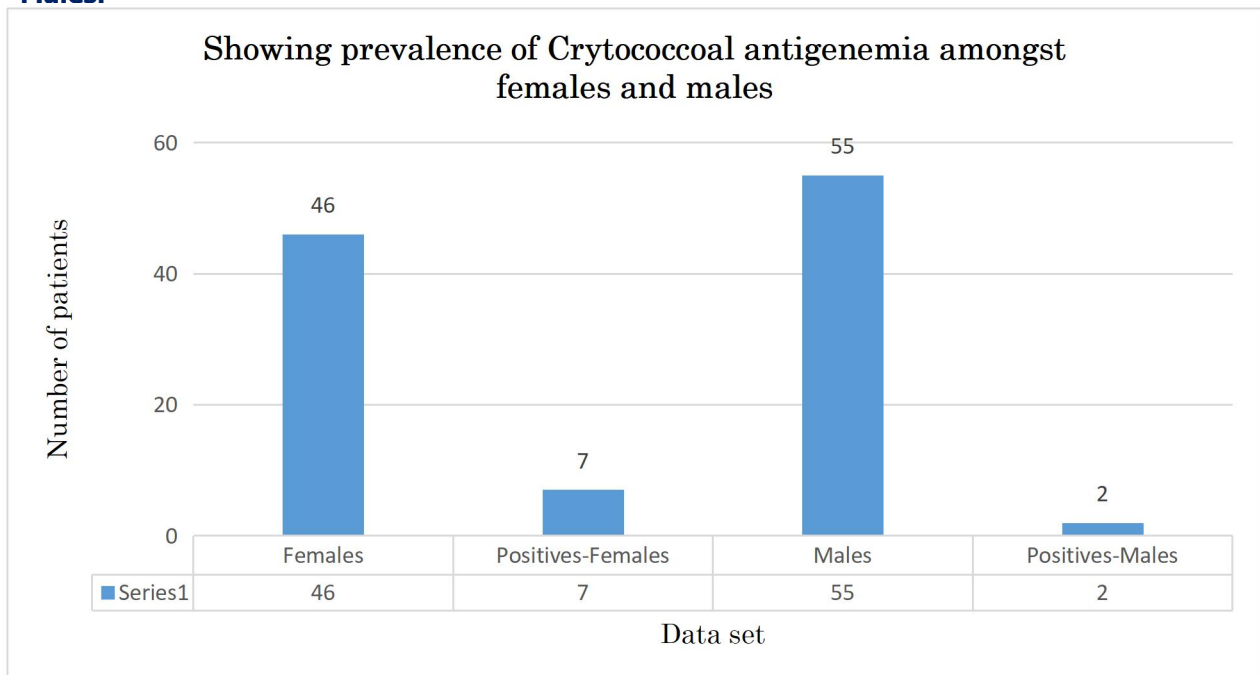
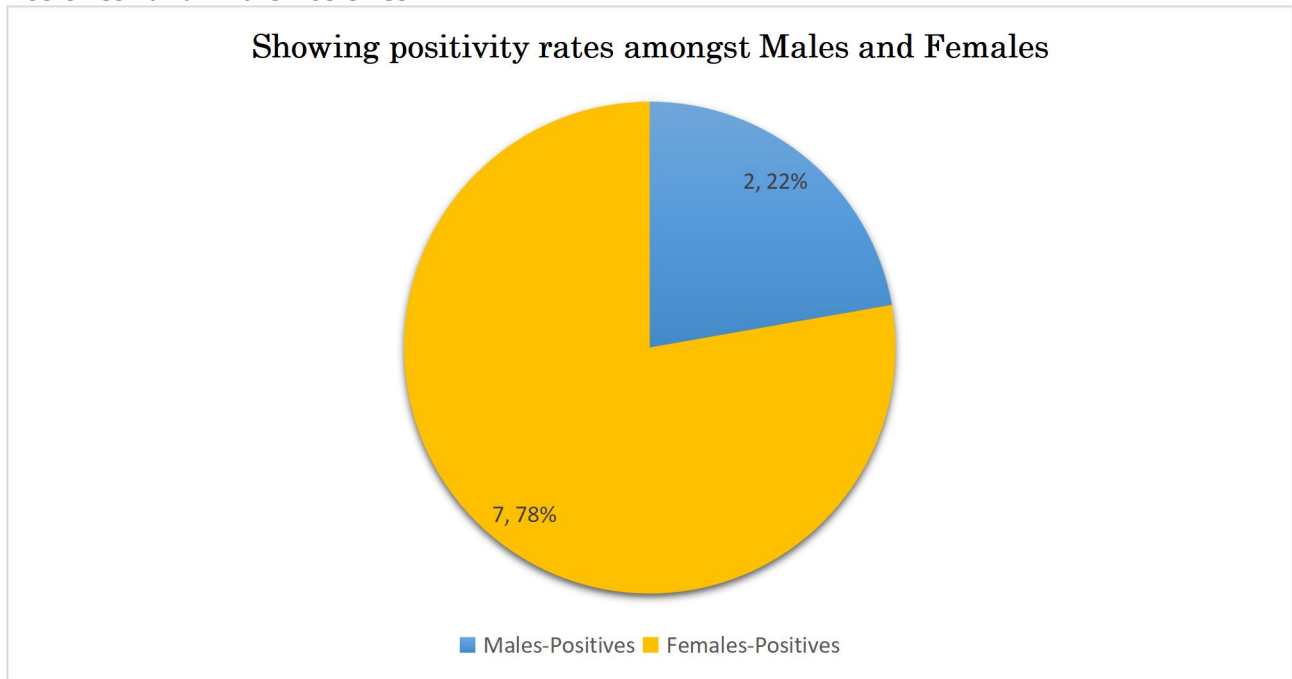


Figure 3 illustrates the positivity rate of patients categorized into two groups: "Female Positives" and "Male Positives."



DISCUSSION

Prevalence of Cryptococcal Antigenemia.

The prevalence of cryptococcal antigenemia among the study participants was found to be 8.91% (9/101). This figure is consistent with previous studies that have reported varying prevalence rates depending on geographical location and patient population characteristics. Notably, our findings revealed a higher prevalence among females (15.2%) compared to males (3.6%), highlighting a gender disparity in susceptibility to cryptococcal antigenemia within our study cohort.

Gender Disparity and Implications.

The observed higher prevalence among females aligns with findings from similar studies conducted in sub-Saharan Africa, where gender-specific differences in immune response and healthcare-seeking behavior may influence disease outcomes. Further exploration into the underlying reasons for this gender disparity could provide valuable insights for targeted prevention and management strategies. Comparisons with other studies underscore the variability in cryptococcal antigenemia prevalence across different settings. For instance, a study reported a prevalence rate of 5% among HIV/AIDS patients in a neighboring region, suggesting regional differences in disease burden that warrant further investigation. Such comparative analyses enhance our understanding of local epidemiological

patterns and inform public health interventions tailored to specific populations.

The identification of a substantial proportion of cryptococcal antigenemia cases underscores the importance of routine screening and early detection among HIV/AIDS patients, particularly those initiating or receiving ART. Early diagnosis facilitates timely initiation of antifungal therapy, potentially reducing the incidence of cryptococcal meningitis and associated morbidity and mortality.

Several limitations should be considered when interpreting our findings. The study's small sample size and single-center nature may limit the generalizability of our results to broader populations within Uganda or other regions. Future research endeavors should involve larger, multicenter studies to validate our findings and explore additional factors influencing gender disparities in cryptococcal antigenemia prevalence.

CONCLUSION

The prevalence of cryptococcal antigenemia was higher in females compared to males among participants aged 15-44 years.

STUDY LIMITATIONS

The study was conducted over a brief timeframe and included a limited sample size, with some clients opting not to participate in the research.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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Page | 6 **RECOMMENDATION**

Regular Immune Status Monitoring by implement regular monitoring of CD4 counts to identify patients at higher risk of opportunistic infections and ensure timely intervention. Developing and implementing gender-sensitive strategies in HIV management to address the higher prevalence of cryptococcal antigenemia among females by considering differences in healthcare-seeking behavior and ART adherence patterns.

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LIST OF ABBREVIATIONS

AIDS:	Acquired Immune Deficiency Syndrome
ART:	Anti-Retroviral Therapy
CD4:	Cluster of Differentiation 4
CrAg:	Cryptococcal Antigen.
HIV:	Human Immunodeficiency Virus
LFA:	Lateral Flow Assay
SOP:	Standard Operating Procedure
WHO:	World Health Organization

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