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SEROPREVALENCE OF *CRYPTOCOCCUS* SP. INFECTION AMONG HIV PATIENTS IN A TERTIARY HOSPITAL IN GHANA

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ABSTRACT

Introduction: *In persons living with HIV, fungal infections contribute significantly to increased morbidity and mortality. Cryptococcal infection is exemplar of invasive opportunistic fungal infections that affect HIV patients. Diagnosis of fungal infections however remains a challenge in Ghana as in many developing countries. Subsequently, there is scanty published literature in Ghana. This can be attributed to the paucity of test assays available for the infection. The purpose of this study was to determine the seroprevalence of the cryptococcal antigen in persons living with HIV in Ghana.*

Methodology: *The study was conducted from August to November, 2018 and included 80 stored serum samples from HIV patients who had reported at the Komfo Anokye Teaching Hospital (KATH), Kumasi, Ghana. Qualitative test was done using the Dynamiker® CrAg Lateral Flow Assay. Results were recorded as positive (++), weakly positive (+) and negative (--). Seroprevalence was also categorized according to patient demographic data as well as HIV treatment statuses.*

Results: *A prevalence of 11.3%was observed, with 9 samples being positive for the antigen and 71 being negative. The study found 10% of patients on ART testing positive, with 13% of ART-naïve samples testing positive for the cryptococcal antigen. There was however no significant association between ART regimen, age or gender and a positive test for the cryptococcal antigen (p=0.05).*

Conclusion: *Approximately 1 in 10 PLWH in this sample had cryptococcal antigenemia, justifying a need for the consideration of routine screening for this invasive fungal disease regardless of antiretroviral treatment status.*

Keywords: *Cryptococcus sp.*, Cryptococcosis, Lateral Flow Assay, Seroprevalence, Human Immunodeficiency Virus, Antiretroviral therapy, Cryptococcal Antigen, Invasive Fungal Diseases

INTRODUCTION

The pathogenesis of the Human Immunodeficiency Virus (HIV) is underlined with the characteristic depletion of the body's immune system – and this aids the propagation of opportunistic infections in infected patients daily; especially in the Sub-Saharan regions of Africa (Agegne *et al*., 2018). An example of such an opportunistic infection is cryptococcosis.

Cryptococcosis is an opportunistic infection caused by the fungi *Cryptococcus sp*. often after colonisation with the encapsulated form of the fungus. Development of cryptococcosis has been often attributed to the species *C. neoformans* (Chayakulkeeree & Perfect, 2008). Once in the immunocompromised human system, the typical anatomical sites for colonization by the pathogen are the meninges, skin and the lungs. *Cryptococcus sp.* is usually acquired by the inhalation of its spores circulating in the atmosphere (El Fane *et al*., 2015). Upon inhalation, the spores settle and disseminate through the body. Typically, these spores are cleared up by the immune system; consequently, only thriving in immunosuppressed and/or immunocompromised patients (Pongsai *et al.,* 2010). The disease conditions are elicited as pulmonary, cutaneous or meningeal (Frimpong & Lartey, 1998) and infections can be symptomatic or asymptomatic indicating or not, evidence of dissemination. Symptoms of the infection are similar in presentation to other infectious diseases which complicate differential diagnoses (Park *et al*., 2009; Rhein & Boulware, 2012). The common complex syndrome of symptoms includes headaches, nausea, fever and fatigue (Hu *et al.,* 2017). Cryptococcal pneumonia is the commonest infection by *Cryptococcus sp.* as the airways serve as the primary conduit for the contraction of the pathogens; but particularly are indistinguishable from all other forms of pneumonia (Brizendine *et a*l., 2011)

Over the past thirty years, the menace of cryptococcosis has gradually progressed as a result of increasing use of immunosuppressive drugs, organ transplant and the spread of HIV (Dambuza *et al*., 2018). Presently, it is one of the leading causes of HIV – mortalities especially in the Sub-Saharan region. Globally, the World Health Organisation (WHO) estimates about 15% of HIV/AIDS – related deaths have been found to be of cryptococcal-origin with an estimated mortality rate of about 81% (181,000 deaths out of 223,000 in 2017) (Dambuza *et al*., 2018). It has been studied that up to 75% of all such mortalities previously mentioned were reported in Africa (Agegne *et al*., 2018; WHO, 2011). Compounding the issue is a myriad of factors which include the paucity of data to support surveillance, delayed and/or unavailable diagnoses as well as treatment for *Cryptococcus sp*. infections. The advancement of modern-day anti-retroviral therapies and prompt diagnoses are playing a critical role in reducing the spread of infections. Unfortunately, especially in Africa, there had not been much emphasis on the use of point-of-care diagnostic tools to improve diagnoses and treatment outcomes for HIV and HIV-related cases within the healthcare system (Ocansey *et al.,* 2019; Dambuza *et al*., 2018).

In Ghana, very little data is available on the situation of Cryptococcosis and this makes surveillance, diagnosis, treatment, methods of administering care and likely preventive methods very difficult (Ocansey *et al.,* 2019). There remain enormous challenges in the diagnosis of HIV-associated cryptococcal

diseases (Sulaiman Lakoh *et al.,* 2023). Conventionally, cryptococcosis is diagnosed by isolating the fungi from a blood or cerebrospinal fluid (CSF) culture and staining them in Indian ink (Morales Lopez and Garcia-Effron, 2021). However, this procedure is generally time-consuming and is less resorted to in diagnoses especially where symptoms are similar to other infectious febrile illnesses. Molecular and serological methods of diagnoses have been recently employed to facilitate more rapid, point-of-care diagnosis of the fungi. These include Polymerase Chain Reaction (PCR), Latex Agglutination (LA) and Enzyme Immunoassays (EIA) and lateral flow assays [LFA] (Lewis White *et al*., 2017; Park *et al*., 2009; Datcu *et al*., 2018). Presently, there is the shift to more point-of-care testing; and this has led to the availability of such kits which have shown reliable sensitivities and specificities in terms of testing. These have included the CrAg lateral flow assay (LFA) from Immuno-Mycologics, Inc. (IMMY; Norman, UK), CrAg LFA (Dynamiker Biotechnologies, China), CryptoPS (Biosynex) and the StrongStrep (Liming Bio). The last two mentioned have been however reported for suboptimal sensitivity and specificity (Kwizera *et al*., 2021).

The aim of this study was therefore to assess the prevalence of Cryptococcal antigenemia using the Dynamiker® Cryptococcal Antigen Lateral Flow Assay. This assay is a dipstick, sandwich immunochromatographic assay, which detects capsular polysaccharide antigens of *Cryptococcus species complex* (*Cryptococcus neoformans* and *Cryptococcus gattii*) in human serum and CSF (Kwizera *et al.,* 2021). Specifically, the study determined the presence of *Cryptococcus sp.* in the serum of HIV-positive patients while also investigating the relationship between *Cryptococcus sp.* infection and anti-retroviral treatment.

MATERIALS AND METHODS

Study site

The investigation was conducted at the Serology Unit of the Komfo Anokye Teaching Hospital (KATH) in Ghana. The hospital is the largest within the Northern sector of Ghana and second largest countrywide; serving as the referral for many other hospitals in the country.

Study population and sampling

The study included a total of eighty (80) serum samples, randomly selected from a pool of archived samples of individuals diagnosed with HIV. The clinical records of selected individuals had no documented history of presence or absence of symptoms of cryptococcal disease. Fifty (50) samples were from patients who were not on antiretroviral therapy (ART-naïve) and the other thirty (30) being from patients who were on ART. The demographic data of these samples were obtained as well as antiretroviral therapy (ART) status. The specific duration of ART for individuals was not obtained and neither was the type of HIV strain.

The Dynamiker® Cryptococcal Antigen Lateral Flow Assay (Dynamiker® Biotechnology (Tianjin) Co. Ltd., China)

The assay is a rapid immunochromatographic test and functions by detecting polysaccharide antigens of the *Cryptococcus sp.* in serum or cerebrospinal fluid (CSF). There are qualitative and semi-quantitative modules of this test available. However, the qualitative assessment was used for this study.

Test for seroprevalence

Each sample was subjected to a qualitative test for the cryptococcal antigen using the Dynamiker® Cryptococcal Antigen Lateral Flow Assay (Dynamiker® Biotechnology (Tianjin) Co. Ltd., China), for Cryptococcal

antigen detection. The LFA results were recorded as positive (++), weakly positive (+) or negative (--). Samples that showed either positive (++) and weakly positive (+) were considered as positive in the final report. Eighty (80) µl of patient serum was pipetted unto the sample pouch of the test kit and the test was made to run for 15-20 minutes according to manufacturer's instructions.

Data analysis

Statistical evaluation was done for this study using the Statistical Package for Social Sciences (SPSS) version 21. Binary logistic regression was used to determine the significance of the independent variables in comparison to the dependent variable.

RESULTS

Demographic data

Results showed that 22.5% of samples were from males while the remaining 77.5% were from females. The age range of sampled patients was between 30 and 70 years with a mean age of 44 years and median age of 43 years. Majority of the samples were from patients of age group 31-40; accounting for 41.3% (33) of all subjects with members of the 61-70 age category having the least numbers accounting for only 8.8% (7/80) of samples. With respect to antiretroviral therapy (ART) status of participants, 50 (62.5%) were ART naïve while the 30 (37.5%) were on ART (Figure 1, 2 & 3).

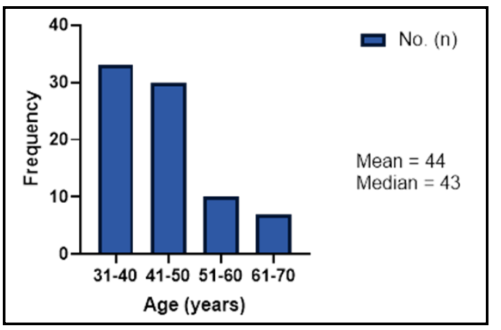

Figure 1: Age distribution of samples

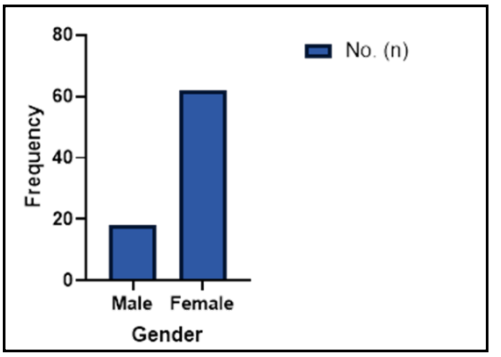

Figure 2: Gender distribution of samples

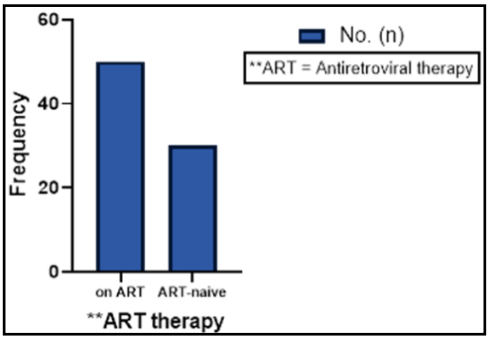

Figure 3: Treatment regimen

Seroprevalence of Cryptococcus sp. antigen

Seventy-one (71) [88.7%] out of the total number of participants tested negative for the Cryptococcal antigen while nine (9) [11.3%] tested positive. Thus, the

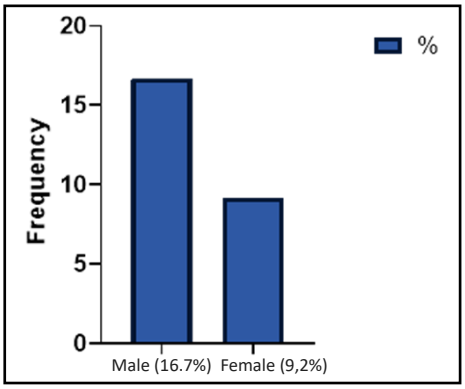

Figure 4: Distribution of seropositivity among gender

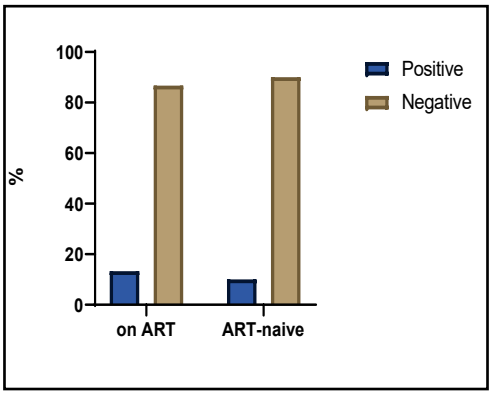

Figure 5: Distribution of seropositivity v. treatment status

prevalence of cryptococcus antigenemia in participant samples determined from this study was 11.3%.

Infection was also found to be present in more females than in males by proportions (figure 4).

A cross-inference to determine the association between antiretroviral treatment and the presence of the cryptococcal antigen in patients showed a prevalence of 13.3% (i.e., 4/30) among participants on ART while determining a prevalence of 10.0% (i.e., 5/50) among ART-naïve patients (Figure 5).

Relationship between Cryptococcus sp. infection and anti-retroviral treatment and demographic factors

A binary logistic regression was conducted to determine any degree of correlation between antigenemia and demographic parameters as well as the antiretroviral therapy status of the samples. It was determined that neither age (p=0.997), gender (p=0.999) nor ART status (0.999) had any significant association with the prevalence of *Cryptococcus sp*. antigen.

Table 1: Binary logistic regression analysis of Cryptococcus sp. infections and suspected predisposing factors

	B	S.E.	Wald	df	*Sig.
Gender	-21.594	16581.272	.000	1	**.999**
Age	-20.705	5922.750	.000	1	**.997**
ART Treatment	19.987	13998.076	.000	1	**.999**
Constant	65.110	24238.560	.000	1	**.998**

**p=0.05*

DISCUSSION

The menace of HIV is being compounded by the number of underlying opportunistic infections which includes cryptococcosis (Agegne *et al*., 2018; Dambuza *et al*., 2018). The findings of this study show the detection of cryptococcal antigenemia in individuals living with HIV in Ghana and this had been seen in studies such as by Mamoojee *et al*. (2011) in HIV patients with CD4 count less than 100 cells/mm^3 and Dyozem *et al.* (2012) who recorded a seroprevalence of 9.86% among a similar population in Africa.

The findings of this study particularly bring to the fore, the need for investigation of cryptococcosis as part of the management and treatment of HIV patients in Ghana. This is important since it is usually one of the least investigated suspected infections among HIV/AIDS patients (together with other invasive fungal diseases) - allowing the disease to progress without surveillance and worse, spread among other susceptible patients. This precedent could have possibly contributed to the prevalence proportions detected from this study.

Given that there was no record of clinical suspicion of cryptococcosis in these HIV patients and yet ~10% of them had the antigen detected, it may be important that clinicians consider screening for cryptococcal antigen in persons living with HIV.

Also, the ability of easy-to-perform tests such as the lateral flow assay in the diagnosis of cryptococcosis could be a critical tool in detecting the antigen – especially compared with clinical examinations for diagnoses which are usually difficult.

Again, the lack of correlation between sociodemographic variables such as age and gender proved to have *cryptococcus* antigenaemia *sp*. (p=0.997 and p=0.999 respectively) may buttress the fact that cryptococcus is only opportunistic and therefore affects all patients due to reduced immunocompetence (Agegne *et al*., 2018, Karaman *et al*., 2019) – a typical characteristic of HIV infection. A similar result was also observed while analysing the effect of ART treatment on seroprevalence of the pathogen. This observation could also be associated with the fact that ART regimen is often aimed at reducing viral loads of the infected patients, and not particularly targeting secondary infections such as with Cryptococcus sp.

CONCLUSION AND RECOMMENDATION

This study realized that approximately 1 in 10 persons living with HIV (PLWH) in this sample had cryptococcal antigenemia; with no particular predilection towards age or gender of affected individuals. This finding reflects what has been observed in the Sub-Saharan region by the WHO and other similar studies; justifying a need for routine screening for this invasive fungal disease regardless of antiretroviral treatment status.

While immunosuppression, clinical stage of disease and duration of AR therapy are some known predisposing factors for cryptococcal disease development in PLWH, this study could not fully investigate such risk factors and thus, it is recommended that further studies be conducted to thoroughly assess such risk factors.

It is also recommended that this study be extended nationwide to facilitate the acquisition of data of the disease among HIV patients and the general populace. Finally, it is recommended that assays be conducted to evaluate the sensitivity and specificity of the cryptococcal antigen lateral flow assay as a step in encouraging routine investigations for *Cryptococcus sp*. among HIV patients.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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