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Prevalence and outcome of asymptomatic Cryptococcal antigenemia in art naive and art experienced HIV patients in Dar es salaam, Tanzania

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PREVALENCE AND OUTCOME OF ASYMPTOMATIC CRYPTOCOCCAL ANTIGENEMIA IN ART NAIVE AND ART EXPERIENCED HIV PATIENTS IN DAR ES SALAAM, TANZANIA

By

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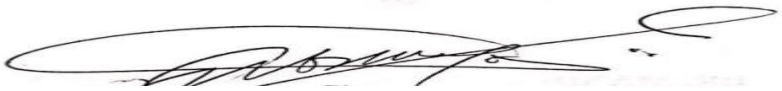

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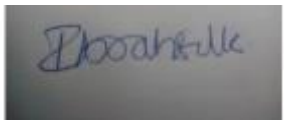

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DEDICATION

I dedicate this work to my parents Ambassador Lt. Gen Charles Makakala and Mrs. Jane Makakala and my wife Dr. Emilia Karugaba for their constant encouragement and overall support.

ABSTRACT

Introduction: Cryptococcal meningitis (CM) is a highly fatal disease and contributes to about 20% of all-cause mortality in HIV/AIDS. Sub-Saharan Africa is facing substantial challenges in the treatment of the disease; the focus has been shifted towards prevention of the condition in ART-naive patients with CD4 counts of less than 100 cells/μL. Most studies and interventions have been conducted on ART-naive HIV patients despite there being cases and deaths due to cryptococcal meningitis among ART-experienced patients. Recent studies have shown that although there is an increase in ART coverage among HIV patients, this has not affected the incidence of cryptococcal meningitis.

Objectives: This study aimed at estimating the prevalence of asymptomatic cryptococcal antigenemia in HIV patients in three HIV clinics in Dar es salaam, Tanzania and to compare the proportion of Cryptococcal antigen (CrAg) positivity between ART-naive and ART-experienced patients, determining factors associated with cryptococcal antigenemia and observation of three months outcomes of patients with cryptococcal antigenemia under recommended treatment.

Methods: This was cross sectional study of HIV positive (ART-naïve and ART-experienced) clients attending three care and treatment clinics in Dar es Salaam from September 2018 to February 2019. Individuals with CD4 count ≤200 cells/μL or viral load ≥1000 copies/ml were screened for serum CrAg. Predetermined three months outcomes; development of meningitis, hospitalization and mortality were assessed between groups.

Results: Two seventy three (273) participants with a mean age of 40.4 years in three HIV clinics in Dar es Salaam were enrolled in this study. The overall prevalence of asymptomatic cryptococcal antigenemia (ACA) was 4.8% (13/273; 95% CI: 2.6 – 8.0). The prevalence in the ART- experienced group was lower 2.5% (4/162; 95% CI: 0.7 – 6.2) compared to 8.1% (9/111; 95% CI: 3.8 – 14.8) in ART-naïve patients ($p<0.05$). Having a history of headache in the past two weeks and hospitalization in the past 12 months were associated with CrAg positivity. Likelihood of death or development of meningitis or hospitalization was higher in CrAg positive patients 69% ($p<0.05$) compared to CrAg negative patients at 12 weeks.

Conclusion: The overall prevalence of asymptomatic cryptococcal antigenemia in ART-naïve and ART- experienced patients is 4.8% in three HIV clinics in Dar es salaam. Prevalence of asymptomatic cryptococcal antigenemia is lower in ART-experienced patients compared to new cases. CrAg positivity is a good predictor of bad outcome at three months.

LIST OF ABBREVIATIONS USED

ACA:	Asymptomatic Cryptococcal Antigenemia
AKU:	Aga Khan University
ART:	Antiretroviral Treatment
ARV:	Antiretroviral
AIDS:	Acquired immunodeficiency syndrome
BMI:	Body Mass Index
BSRF:	Blood sample Report Form
CA:	Cryptococcal antigenemia
CD4:	Cluster of differentiation 4
CTC:	Care and treatment clinic
CrAg:	Cryptococcal Antigen
CRF:	Case report Form
ICF:	Informed Consent Form
IQR:	Interquartile range
WHO:	World health organization
HIV:	Human immunodeficiency virus
LFA:	Lateral Flow Assay
OPD:	Outpatient department
OR:	Odds Ratio

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First of all, I give thanks to the Almighty God for His blessings and guidance.
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I am also thankful to all the study participants for taking their time to consent and take part in this study
My gratitude also goes to my research assistants for their invaluable support.
Thank you all.

DECLARATION

I declare this dissertation does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university and that to the best of my knowledge it does not contain any material previously published or written by another person except where due reference have been made in the text.

(Signature of candidate)

14th June 2019

Date

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OPERATIONAL DEFINITIONS

For purposes of this study, the following operational definitions were used:

ART-experienced patient: HIV positive patient who has used ART for more than 6 months.

Asymptomatic cryptococcal antigenemia (ACA):

Is individual who has positive serum CrAg without symptoms of cryptococcal meningitis (not more than one of the following; headache, fever, neck stiffness, altered mentation, convulsions, photophobia/blurred vision)

ART-naive patient: HIV positive patient who has never used ART before or has used ART for a period equal or less than six months

Recent CD 4 Count: CD4 Count of not more than three months old

Recent Viral load: Viral load of not more than three months old

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1.0 BACKGROUD

1.1 Introduction

Cryptococcus infection, especially in immunocompromised states is mostly caused by the fungus Cryptococcus neoformans (C. neoformans)(1). Recently, species such as Cryptococcus. Gatii (C. gatii) has been associated with Cryptococcus meningitis in immunocompetent patients in North America (2). The natural history of the disease starts when a subject inhales desiccated yeast of Cryptococcus species. The yeast are engulfed by alveolar macrophages and in most cases, the infection is cleared (1). In immunocompromised states like HIV/AIDS, the yeast reproduce and form spores causing infection to be disseminated to the bloodstream and eventually crossing the blood-brain barrier to cause meningitis(3). A patient may be asymptomatic during the initial stages of the disease; during the later stages of the disease, dissemination and inflammation in the brain and meninges will cause symptoms of meningitis. Such symptoms include fever, headache, altered mental state and visual problems(4).

Cryptococcal meningitis accounts for 20% of mortality associated with HIV globally. It is estimated that there are about 220,000 cases of cryptococcal meningitis annually that results to 181,000 deaths. Sub-Saharan Africa contributes to majority of the cases approximately 162,500 cases per year (5).

This infection is one of the neglected tropical diseases; In 2015 on drug development research, it accounted for1% of fund used in Tuberculosis(6). Cryptococcal neoformans is the leading cause of meningitis in the adult sub-Saharan population including Tanzania (7,8). The disease itself has high case fatality rate 35-65% in low and middle-income countries and 15- 20% in high income country (9,10).

There are lots of challenges in the diagnosis and treatment of cryptococcal meningitis especially in developing countries. The scope of challenges includes; lack of diagnostic tools, availability of medication (flucytosine, amphotericin B), safety of amphotericin B and high cost of treatment as well as shortage of physicians able to perform lumbar puncture(9,11,12). Even with proper treatment and increasing ART coverage, the prevalence and mortality rate of cryptococcal meningitis is still high, therefore, the focus is now shifting to preventing patients with low CD4 (<100) from developing the disease (10). Much focus has been directed in screening and

preempting treatment of asymptomatic cryptococcal antigenemia (ACA) in HIV patients with CD4 cell count of less than 100/ µL (13).

Asymptomatic Cryptococcal antigenemia (ACA) is defined as the presence of cryptococcal species in the blood without having symptoms of meningitis; fever, headache, stiff neck and altered mental status. This is a subclinical disease which may progress to Cryptococcal meningitis within 3-6 weeks if left untreated. Despite recommendations of WHO guideline of screening for cryptococcal antigenemia in ART-naïve HIV patients, only about 20 countries in the world have adopted the recommendations (6). Tanzania, for example, is yet to formally include CrAg screening in her care of people living with HIV.

1.2 Problem Statement

Cryptococcal meningitis remains one of major cause of mortality in HIV patient even in Post Antiretroviral Treatment (ART) era. Lack of resources in sub-Saharan Africa results in more mortality of cryptococcal meningitis in this region. Most patients with cryptococcal infection have subclinical disease at onset therefore screening offers early diagnosis and early treatment of disease.

There is an epidemiological evidence of screening for cryptococcal antigenemia in ART- naïve patients with CD4 count of <100 cells/µL and preempting treatment of patients with positive asymptomatic cryptococcal antigenemia. This recommendation has been adopted in most HIV treatment guidelines in Sub-Saharan Africa countries including Tanzania. However, this has not been very effective due to multiple factors such as availability of CD4 count testing at diagnosis, coverage of CrAg screening tests in the routine tests.

HIV patients with CD-4 count higher than 100 cell/µL are not currently been screened for cryptococcal antigenemia and may be missed with the current guideline recommendations. Some studies have demonstrated Cryptococcal meningitis cases in CD-4 count higher than 100 cell/µL.

Currently, there are no recommendations and guidelines on screening for Cryptococcal antigenemia in ART experienced patients. Although there is an increase in coverage of ART among patient living with HIV, this has not significantly reduced the number of deaths related to

cryptococcal meningitis. This could be related ART treatment failure. There is lack of data on screening of ART- experienced patients.

1.3 Study Justification

Due to change in HIV treatment guidelines, invariably, all ambulatory newly diagnosed HIV patients are started on antiretroviral therapy regardless of their CD-4 count. Since adoption of this new guideline no study has been done in Tanzania to find how that affect screening of cryptococcal infection in newly diagnosed HIV patients.

There is lack of data on screening of cryptococcal antigenemia in ART experienced patients with treatment failure and the magnitude of asymptomatic infection in this group is not known. Some hospital based studies have described higher prevalence of cryptococcal meningitis in ART experienced patients compared to ART- naïve patients. From the literature there is no significant reduction of mortality related to cryptococcal meningitis in the post ART era compared to pre ART era. Viralogical failure is the inability to achieve or maintain suppression of viral replication to an HIV RNA level less than 1000 copies/ mL, patient with viralogical failure are at risk of HIV/AIDS clinical progression. Currently there is no study that have determine prevalence of cryptococcal infection in HIV patients without viralogical suppression.

Recent studies have demonstrated cryptococcal meningitis cases in HIV patients with CD4 count higher than 100 cell/µL. This study will use a cut of point of CD4 count less than 200 cell/µL.

This study has looked at prevalence of asymptomatic cryptococcal in ART-experienced patients without viralogical suppression. The present study has included HIV patients with CD-4 count of equal or less than 200 cell/µL in order to provide an outlook of ACA in this cohort.

2.0 LITERATURE REVIEW

2.1. Microbiology of cryptococcal infection

Cryptococcus is basidiomycetous, encapsulated yeasts; it has two species C. neoformans and C. gattii. The two species are further subdivided into 4 serotypes depending on the polysaccharide antigens that compose the capsule; A and D under neoformans species and B and C under gatii species (13). Serotype A (variety grubii) is also subdivided into three molecular subtypes VNI, VNII, and VNB (13).

C. neoformans reproduce both sexually and asexually; the asexual forms are the ones that can be isolated from human infections and they produce by budding (14). The sexual forms have been observed only in the laboratory; the yeast forms has two mating types named α and a. Conjugation between these types forms teleomorph with hyphae.

Some of the hyphae specialize in a structure called basidia where meiosis occurs to form uninucleate, unencapsulated basidiospores. After they have detached from the basidia they quickly start to form capsules. The budding process only occurs on the encapsulated spores to complete the life cycle (14).

The spores' structure contains two components important in the virulence; the polysaccharide capsule and melanin (15). The capsule can be visualized under a microscope in a suspension of India ink, it varies in size but can contribute up to half of the diameter of the spore. The capsule is antiphagocytic. The larger the capsule the more virulent is the yeast as observed in animal models (16). The capsular polysaccharide antigens make the basis of the current test kits of CrAg (13).

The spore's cell wall produce melanin which is an antioxidant that accumulates and protects the yeast against oxidative products and immune cells. *C. neoformans* has an enzyme called phenoloxidase that catalyzes phenolic substrates such as dopamine and epinephrine. High level

of dopamine in the central nervous system gives the organism a survival advantage in that environment through the production of large quantities of melanin (17).

Cryptococcus neoformans is found in soil samples from around the world, especially in environments with birds like pigeons and chickens. The pigeons do not become infected because of their elevated body temperature (>40ºC). Also, the fungus has been linked with areas of rotting vegetation (18).

2.2 Human cryptococcal infection

Humans get infected by inhaling the basidiospore which is small desiccated yeast with small polysaccharide capsule which makes it easy to be deposited in the alveoli and terminal bronchioles (18).

In immunocompetent individuals the yeast is engulfed by macrophages forming a small granuloma/hilar lymphadenopathy, in most of the cases the infection is cleared(19) . Some patient develops symptomatic focal pneumonitis presenting with a cough, sputum production, hemoptysis, dyspnea, chest pain, fever, malaise, night sweats, and weight loss (20). Less commonly the patients may have a rash and gastrointestinal symptoms and very rarely superior vena cava obstruction and Pancoast syndrome due to a granulomatous extension to the apex of the chest wall (21).

In Immunocompromised individuals such as stem cell and solid organ transplantation, HIV infection, malignancies, cirrhosis, renal failure, chronic lung, Cushing's syndrome, sarcoidosis, and treatment with glucocorticoids or tumor necrosis factor-alpha antagonists reactivation of latent infection may occur and result in disseminated extra pulmonary infection to the skin and central nervous system (22,23).

Cutaneous manifestations are seen in 15% of patients with disseminated infection; which present as papules, plaques, purpura, ulcers, cellulitis, superficial plaques, abscesses, and sinus tracts (24).

Dissemination to the central nervous system will cause inflammation of the meninges, brain matter and granuloma formation in the brain parenchyma resulting in acute and subacute meningoencephalitis symptoms like fever, headache, vomiting, altered mentation and convulsions (22).

2.3 Immunology of cryptococcal neoformans

The understanding of how the human body immunity reacts to cryptococcal infection is vital because it can guide treatment strategies and predict severity and prognosis of infection. There is a difference in immune activation of cryptococcal neoformans between HIV positive and HIV negative individuals (25).

After dissemination of the spores in the bloodstream, the fungal mannoproteins are recognized by dendritic cells through toll-like receptor 2(TLR2) that present the antigen to T-cells. In HIV individuals there is a direct linkage between the activated memory T- cells with the peripheral blood mononuclear cells (PBMC) (5).

Due to very low CD4+ T cells in HIV the individuals the immune system makes memory T- cells without exaggerated immune response. When the patients are initiated on ARV CD4+ cell increase and are activated through the memory cell to produce cytokines mostly interleukin-6(IL6), Interferon gamma (IFNγ) and TNF causing cryptococcal immune reconstitution syndrome (*c*IRIS) (5).

But because there is a direct synapse of Activated T-cells and macrophages in HIV positive individuals this results in a better antigen clearance compared to HIV negative individuals. In contrast, the HIV negative individuals with the cryptococcal infection present with a similar inflammatory response just after acquiring the infection termed as Cryptococcal post-infectious immune response syndrome (*c*PIIRS) (5).

2.4. Association between Cryptococcal Antigenemia and CD4 Count

Many studies have been done on cryptococcal antigenemia in HIV/AIDS patients, Cryptococcal antigenemia was found to be more common if the patient had CD4 count of less than 100 cells/ µL. However several studies have also demonstrated cases of Cryptococcal antigenemia at higher CD 4 counts.

In a study done by *Favour et al* in Benin Nigeria that screened 150 ARV naive patients of whom 19(13%) patients had cryptococcal antigenemia Seventeen (89%) of the 19 patients had CD4 cell counts less than 100 cells/µL and the remaining two had CD4 counts between 100 and 200 cells/µL (26). Most patients in this study with positive serum CrAg had a CD4 count of less than 50cells/µL. The prevalence in this study was substantially higher than other countries but still shows that there are cases that can be missed in a group of patients with CD4 count between 100 -200 cells/µL.

In a retrospective study done in Cape Town, South Africa involving 707 HIV patients 92% of patients with serum CrAg-positive had CD4 cell count less than 100 cells/µL and 8% with CD4 cell counts between 100 and 200 cells/µL (27).

In a cohort study done in Northern Tanzania among 333 hospitalized ARV naive HIV patients, 17(5.1%) patients had serum CrAg-positive. Among these 17 patients, the median CD4 count was 68 cells/mm3 (IQR 41-87, range 1-102 cells/µL) (28).

In a crossectional study conducted Ethiopia among 254 HIV patients both ARV naive and ARV experienced; Serum CrAg positivity was 20.9% in persons with CD4 count 150 cells/µL, 12.2% in 151–200 cells/µL, 5.8% among 201–350 CD4/µL, and none above 350 cells/mL. This study found a substantial number of CrAg positive patients with CD4 count between 100-200 cell/ µL (29).

In these studies, there is a positive correlation between serum CrAg positivity with the CD4 count less than 100cells/µL because more cases of cryptococcal antigenemia had CD4 count of less than 100 cell/ µL. WHO guideline for the treatment of cryptococcal meningitis recommend screening for serum CrAg for newly diagnosed HIV patients with the CD4 count less than

100cells/µL (30). However isolated studies have shown small percentage of patients with higher CD4 cell counts with positive serum CrAg and there for by using current screening guidelines some cases will be missed.

2.5 Prevalence of asymptomatic cryptococcal antigenemia

A number of studies have been done to find the magnitude serum cryptococcal antigenemia in HIV patients with low CD4 count however only few were done specifically on an asymptomatic/preclinical phase of cryptococcal meningitis. Most of these studies were in African countries.

In one review article, the prevalence of asymptomatic cryptococcal antigenemia ranges 6-13% among patients with the CD4 cell counts less than 100/µL, although there were a lot of geographical variabilities (4). Cryptococcal antigenemia is 100% sensitive in predicting the development of cryptococcal meningitis (27).

A hospital-based cross-sectional study conducted in Uganda among 367 ART-naive patients with CD4 cell counts of less than 100cells/µL found a cryptococcal antigenemia prevalence of 19%. However, this study did not exclude those patients with symptoms of meningitis and it only included ART-naive patients (31).

In a clinic-based study carried out in Indonesia among 810 ART-naive patients with the CD4 cell counts of less than 100cells/µL with no symptoms or signs of meningitis; the prevalence of ACA was found to be 7.1% (32).

In Benin, Nigeria a crossectional study of 150 ART-naive patients from out-patient department with CD4 cell counts of less than 200 cells/µL the prevalence of cryptococcal antigenemia was 12.7% (26).

In a retrospective cohort done in London on 157 ART-naive patients, the prevalence of cryptococcal antigenemia was 5% this study included patients with symptoms and signs of

meningitis (33). Different kind of results was observed in Ethiopia among 129 ART-naive patients where the prevalence of cryptococcal antigenemia was only 1.6% which was somewhat low compared to other studies in the region (34). All these studies did not exclude patients with symptoms of meningitis.

Another study was conducted in Ethiopia that compared the prevalence of cryptococcal antigenemia between ART-naive and ART-experienced among 254 patients with CD-4 cell counts of less than 350cell/μL. The prevalence of cryptococcal antigenemia was 10.2% overall, 14.2% among ART-naive, 4.1% among ART-experienced, and 50% (3/6) among ART-defaulters, regardless of CD4 cell counts (29).

Another study performed in the same country among 369 HIV patients found a prevalence was 8.4%, among which 84% with CrAg positive were ART-experienced. This poses importance of screening the ART experience patients with CD4 of less than 200 cells/μL (35).

In Namibia, 181 ART-naive patients with CD4 cell counts of fewer than 200 cells/μL were screened for cryptococcal antigenemia prevalence of 3.3% was obtained (36).In a larger crossectional study in South Africa among 707 ART-naive patients, the prevalence of asymptomatic cryptococcal antigenemia was 7% (n=46) and cryptococcal antigenemia was 100% sensitive in predicting the occurrence of cryptococcal meningitis during the first year of ART (37).

Another study was done in the middle east in Iran among ART naïve patients with CD4 count less than 100 cells/ μL and the prevalence of cryptococcal antigenemia was found to be 0% unlike other regions(38).

In Tanzania, there are three studies conducted about cryptococcal antigenemia; 333 HIV hospitalized patients were screened for CrAg where 4.4% were positive (28). Another study done in the northern zone revealed a prevalence of cryptococcal antigenemia was 8.3% (12). Both of these studies were done in hospitalized and symptomatic patients.

In Another retrospective study done in Central rural Tanzania in 2012, looking at one year outcome of the cryopreserved blood of 801 HIV – ART-naive patients with CD4 cell counts less

than 150 cells/µL, 29 (3.9%) were CrAg positive. Among which 34% had symptoms of meningitis and 66% were asymptomatic (36).

In review of these studies, there may be a geographical variation of prevalence of cryptococcal antigenemia. Many studies were done in ART naïve patients, most studies were retrospective and CrAg was tested in cryopreserved samples. Very few studies were looking at prevalence of asymptomatic cryptococcal antigenemia.

2.6 Associated factors with cryptococcal antigenemia and short-term outcome

From the literature, it is well established that having low CD 4 count is a risk factor for acquiring the cryptococcal infection. Several studies have looked at Factors associated with cryptococcal antigenemia, one of the significant associating factors was BMI less than 18 kg/m2 (p=0.0007), but anemia, high viral load, and active TB were not significantly associated (39).

BMI of less than 15.4 Kg/m2, being male, rural residence, and being hospitalized were associated with cryptococcal antigenemia (31,34).

One study in Nigeria looked on current ART regimen association with CrAg positivity, but no factors associated with cryptococcal antigenemia was determined (40). The large cohort in Indonesia looked at additional factors like WHO Clinical stage, co-infections, laboratory values but no significant factors associated with cryptococcal antigenemia where determined (32).

A retrospective study done in northern Tanzania among ART-naive patients with CD4 cell counts fewer than 200 cells/µL with positive CrAg and in which patients received preempting treatment showed no significant difference in mortality among CrAg positive and CrAg negative patients after six months (12).

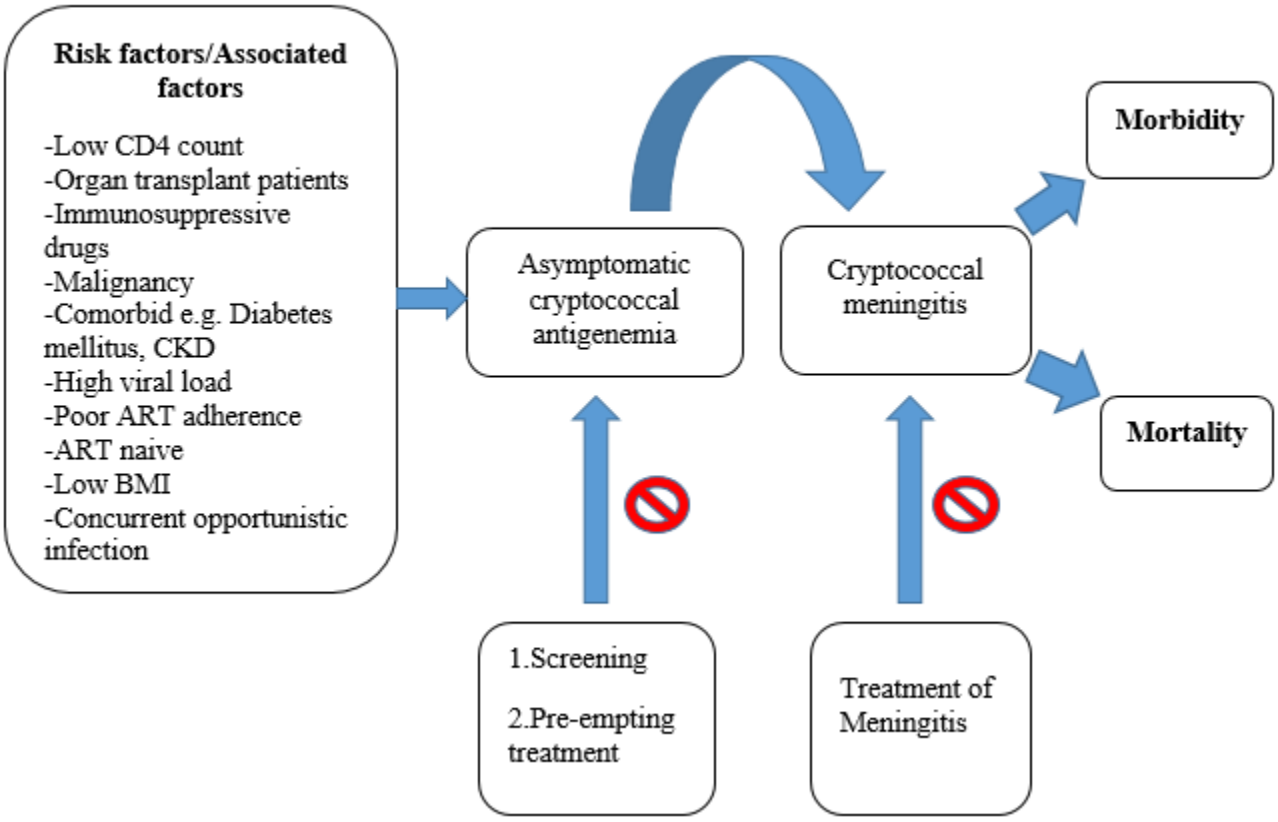
In Another retrospective study done in Central rural Tanzania in 2012, looking at one year outcome of the cryopreserved blood of 801 HIV – ART-naive patients with CD4 cell counts less

than 150 cells/µL, 29 (3.9%) were CrAg positive. Among which 34% had symptoms of meningitis and 66% were asymptomatic. One year Mortality between patients who had symptoms of meningitis was not different compared to those who were asymptomatic 70 and 74% (p=8) respectively (36).

Asymptomatic cryptococcal antigenemia was found to independently predict death during the first 3 months of ART among individuals with advanced HIV disease in rural Uganda. In this study 23% of patients with CrAg positive died within the period of three months (39).

Figure 1. The conceptual framework.

Describing several risk factors that predispose a patient to cryptococcal infection. The framework also illustrates factors that are associated with asymptomatic cryptococcal antigenemia, interventions that reduce development to cryptococcal meningitis which are mainly screening for asymptomatic cryptococcal antigenemia and preempting treatment with fluconazole for CrAg positive patients. With effective intervention this will reduce mortality and morbidity associated with cryptococcal meningitis.



3.0 OBJECTIVES

3.1 Research Question

What is the prevalence and the outcome of asymptomatic cryptococcal antigenemia in ART-naïve and ART-experienced HIV patients in Dar es salaam, Tanzania?

3.2 Primary Objective

To determine the prevalence of asymptomatic cryptococcal antigenemia and development of meningitis in three HIV clinics among ART-naive patients with CD4 count $\leq$ 200 cells/µL and ART-experienced patients with HIV viral load of $\geq$ 1000 copies/ mL

3.3 Secondary Objective

1. To determine factors associated with asymptomatic cryptococcal antigenemia among HIV patients.
2. To determine short-term outcomes (development of meningitis, deaths, number of hospitalization) between ART-naïve/New cases and ART-experienced patients with asymptomatic cryptococcal antigenemia.

4.0 METHODS

4.1 Research Design

The study design is cross-sectional, clinic-based study. Study participants who fit the inclusion criteria were tested for serum Cryptococci antigen. Participants with positive CrAg were referred to their treating physician with a recommendation for preempting treatment according to WHO guidelines. CrAg-positive patients were followed up and at the 12th week from the date of enrolment; short-term outcomes (Death, Hospitalization, and Development of symptoms of meningitis).

Study variables

Table 1. Showing depend and independent variables

Dependent variables	Independent variables
CrAg LFA status	CD-4 cell count Viral load BMI History of hospital admission in the past 12 months Duration of HIV treatment Co-existing HIV associated illness ART Adherence Cryptococcal meningitis(development of at least 2 symptoms of meningitis) Death Hospitalization

4.2 Study Location

Currently, there are 1.4 million people leaving with HIV in Tanzania; this accounts for a prevalence of 4.7% and there are 55,000 new infections per year (41).This study was conducted in a metropolitan city of Dar es Salaam which is the highest populated city in Tanzania. According to the 2012 national census the region had a population of 4,364,541 people but it is projected that the population is around 5,502,000 in 2017. With a prevalence of 4.7%, it is estimated that Dar es salaam has approximately 258,594 people living with HIV (42).

The city is divided into three administrative districts each with district hospital namely Temeke, Mwananyamala and Amana Hospital. There are also many private hospitals; among which is Aga Khan Hospital is situated in Ilala district. Mnazi mmoja hospital is a public hospital situated at the city center and has one of the largest HIV clinic in Dar es Salaam.

All hospitals whether public or private operate an HIV Clinic (CTC clinic) that is completely run by the ministry of health of Tanzania. New patients are registered in specific clinics in which they receive treatment and are being monitored. Demographic information, patient contacts, TB screening, CD4 cell count and viral load test are usually taken at beginning of the treatment. Follow up is done monthly, and viral load annually to monitor treatment (43).

Participants from three CTC clinics were recruited for this study; Mwananyamala hospital and Mnazi Mmoja hospital which are government institutions and Aga khan hospital which is a private Hospital. Multiple sites were purposefully chosen in order to have adequate representation of Dar es Salaam and to facilitate attainment of the minimum sample size within the short study period.

4.3 Study population

Study participants were HIV patients (ART- naïve and ART- experienced) attending the HIV clinics in Aga Khan, Mwananyamala, and Mnazi Mmoja. This sites are care and treatment clinics (CTC) that are administratively run by ministry of health of Tanzania as all other CTC centers in Tanzania. The centers provide free HIV service and management of both adult and pediatric HIV patients. Mwananyamala and Mnazi Mmoja CTC are under public hospital and are one of the largest HIV clinics receiving patients from all parts of Dar es salaam city. The Aga khan CTC is under a private hospital. The patients that attend these clinics are good representative of patient attending other CTC clinics in Dar es salaam.

Inclusion criteria

- Adult (≥18 years) HIV positive patients attending HIV clinics in Dar es Salaam recent CD4 cell counts ≤ 200cells/μL.
- ART-experienced patients (On ART treatment more than 6 months) with viral load ≥ 1000copies/ml

Exclusion criteria

- Patients with two or more symptoms of meningitis (fever, headache, stiff neck, altered mental status)
- Patients on fluconazole or has been on fluconazole in the past two weeks
- Patients on cryptococcal meningitis treatment or had the cryptococcal infection in the past year.
- Those who could not be followed up for three months

4.4 Sample size calculation

The prevalence of cryptococcal antigenemia from a study conducted in 5 HIV clinics in Dar es Salaam was found to be 5%. But this study was done in ART-naive patients only; this prevalence was same compared with similar studies done in Indonesia and South Africa where the prevalence where 7.1% and 7%. But the study done in Ethiopia that included both ART naïve and ART experience patients the prevalence of cryptococcal antigenemia was 10.2%, another study on the same country showed 84% of patient with positive CrAg were HIV experienced. The prevalence of 10.2% fall within the stated mean prevalence of ACA in review article which is 6-13%, the study used 10.2% as the expected prevalence in our set up. Sample size is estimated using the using a formula for estimating population prevalence.

$$\mathbf{n = (Z^2 \times P\,(1 - P))/e^2}$$

Where Z = value from standard normal distribution corresponding to desired confidence level (Z=1.96 for 95% CI)

P is expected true proportion

e is desired precision (half desired CI width).

$$n = (1.96^2 * 0.102(1-0.102))/0.04^2$$

$$n = 220$$

With an assumed 20% attrition rate due to loss of follow, the minimum sample size of 265 participants was be recruited for the study.

Sampling Procedure

A convenient sampling technique was employed. All patients who attended HIV clinics who met the criteria were included in the study. There was no predetermined distribution of sample size between the three clinics; therefore the number contributed by each clinic depended on how quickly the recruitment process was completed in that clinic. Study participants were recruited until the minimum sample size was reached.

All the CrAg positive participants were followed up at twelve weeks from date of recruitment. At the time of analysis, not all of the CrAg negative participants had finish twelve weeks from date of recruitment so simple random sampling was conducted to select a sample of 30 participants to make a ratio of CrAg positive to CrAg negative 1:2 for comparison of outcomes at twelve weeks.

Research tools

A lateral flow assay (LFA) from IMMY Biotech Company was used to screen for cryptococcal antigenemia. The assay has been shown to have more sensitivity (95-100%) and specificity (100%) over traditional latex agglutination. It is also simpler to use, has a short turn over time (10 minutes) and cheaper (31). The WHO guideline has recommended LFA technique in the screening of cryptococcal antigenemia.

The results for CrAg and titers were recorded on a blood sample report form (BSRF). At 12 weeks participants/next of kin were followed up through a phone call and asked about the development of symptoms of meningitis, hospital admission, and death. The responses to the questions were recorded on a structured questionnaire.

Data Collection Procedure

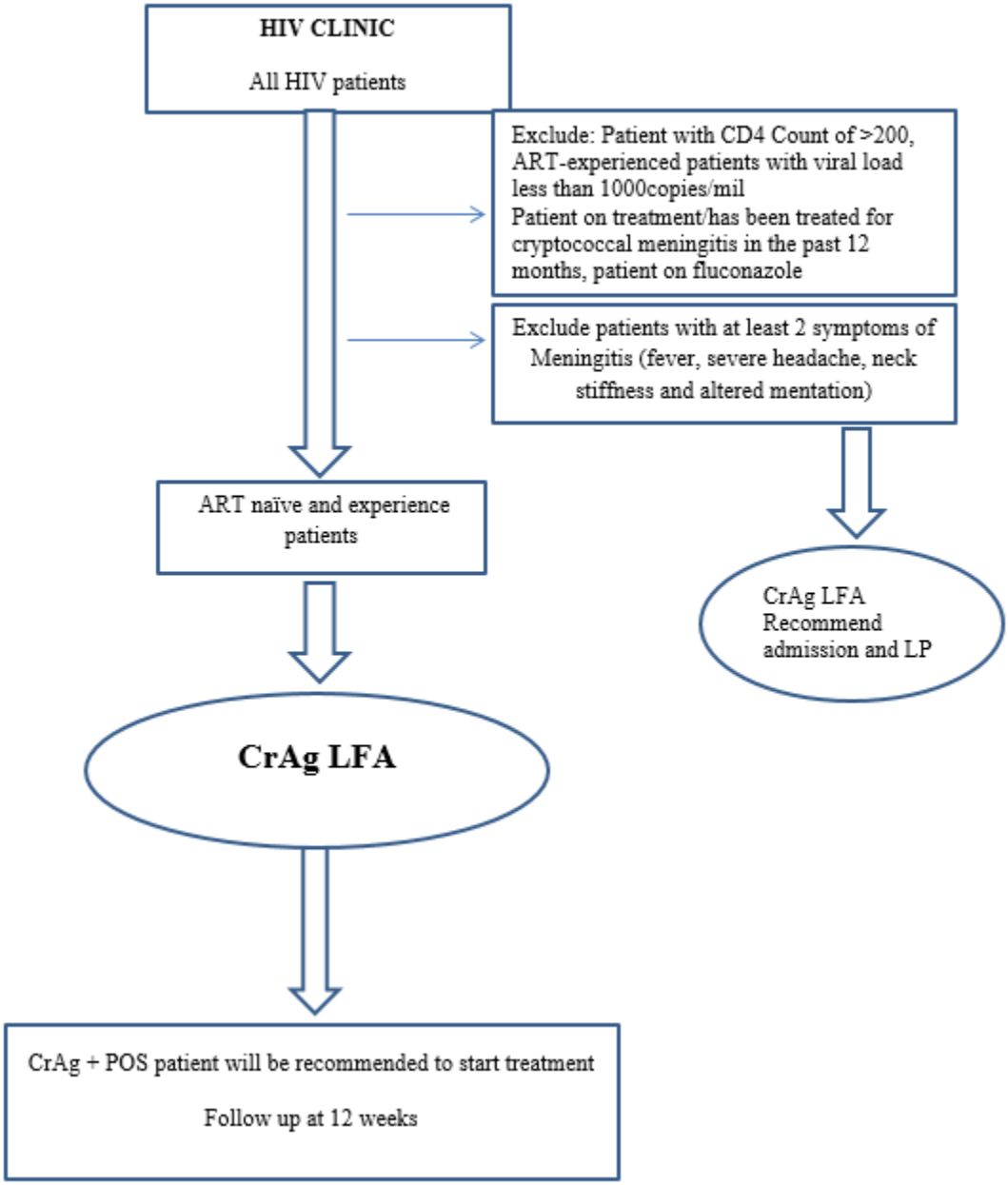
The Information was collected from participants through an interview and patient follow up file; this information was recorded on a case record form (CRF) and later entered in an electronic data management system. The variables collated included age, sex, weight, height, BMI, date of HIV diagnosis (duration of HIV infection), date of ARV Commencement (duration of ARV use), current

ARV regiment, recent CD4 Count (less than 3 months old), recent viral load, WHO clinical staging, opportunistic infection/AIDS-defining illness; and comorbidities such as history diabetes mellitus, malignancy, renal failure, and hypertension, history of concurrent medication was also obtained. Vital status (dead or alive) of the patients was also collected at 12 weeks of follow-up.

Age was recorded in years, gender as female or male, weight was measured with a normal well calibrated outpatient weighing scale and recorded in Kilograms, height was measured in centimeters, CD 4 count was recorded per milliliters (ml), viral load was recorded in copies/milliliters, duration of HIV treatment was recorded in months.

Two microliters venous sample was drained and screened for cryptococcal antigenemia using CrAg – LFA rapid test. All patients who were positive for CrAg were referred to their treating physician for treatment as per WHO guideline for preempting treatment of cryptococcal meningitis. For every 1 CrAg positive patient, 2 CrAg negative patients were followed up for 12 weeks since the date of enrolment and assessed for death, hospitalization, and development of symptoms of meningitis.

Figure 2. Study Protocol flow diagram



4.5 Data Management

Data was collected with the case report forms and Data entry form was generated in Epi info 7 Build 7.2.2.16 (2018) and data was entered in statistical package. The investigator reviewed the entire questionnaire to ensure that there were no transfer errors. Data was stored in a backup drive with password protection to ensure the security of data. During the end of the study, all original data was submitted to AKU Faculty of Health Sciences as per requirements of the university.

4.6 Data Analysis

Data was analyzed using Epi info 7.

Baseline characteristics were summarized on a table as nominal data. Univariate analysis of factors associated with ACA was done, Chi Square test (Fishers Exact test where appropriate) as well as student t-test were used to determine difference between categorical or continuous variables respectively.

The prevalence of asymptomatic cryptococcal antigenemia was determined as percentage of who screened positive for Cryptococcal serum antigen (CrAg) from total study sample. Chi Square test was used to assess the difference in prevalence between ART-naïve and ART-experienced cases.

Odds ratio (ORs) with 95% confidence intervals (CI) were calculated to draw association between variables. All factors that were statistically significant in univariate analysis were analyzed with Multivariate logistic model to control cofounders. The level of statistical significance was taken to be p-value of <0.05.

Outcomes (Development of meningitis, hospitalization, mortality) after follow-up period of 3 months were describes as percentage. And comparison of likelihood of bad outcome between CrAg positive and CrAg negative patients was done with chi square test.

4.7 Ethical Considerations

Ethical approval was obtained from Ethics and Research Committee of The Aga Khan University and the National Institute of Medical Research Tanzania. Written Permission from institutions participating in the study was obtained. Informed written consent was obtained from all study participants who consented to participate in this study. Participants were informed that the information they provided would be encrypted with specific numbers for confidentiality and their names would not be disclosed. The study did not interfere with the management of the patients. All CrAg positive patients were returned back to their treating Doctors for management. The study advised preempting treatment for cryptococcal antigenemia according to WHO guidelines. Blood samples collected were used only to test for cryptococcal antigen and then discarded.

5.0 RESULTS

From September 2018 to February 2019, a total of 273 participants were enrolled into the study from three different CTC clinics Mnazi Mmoja, Mwananyamala and Aga khan. Figure 3 shows summary of study participants who met the inclusion criteria and consented for the study (convenient sampling method was employed, therefore not all patient attending the clinic were assessed for the study) and Table 1- Baseline characteristics of study participants.

Figure 3. Summary of study participants

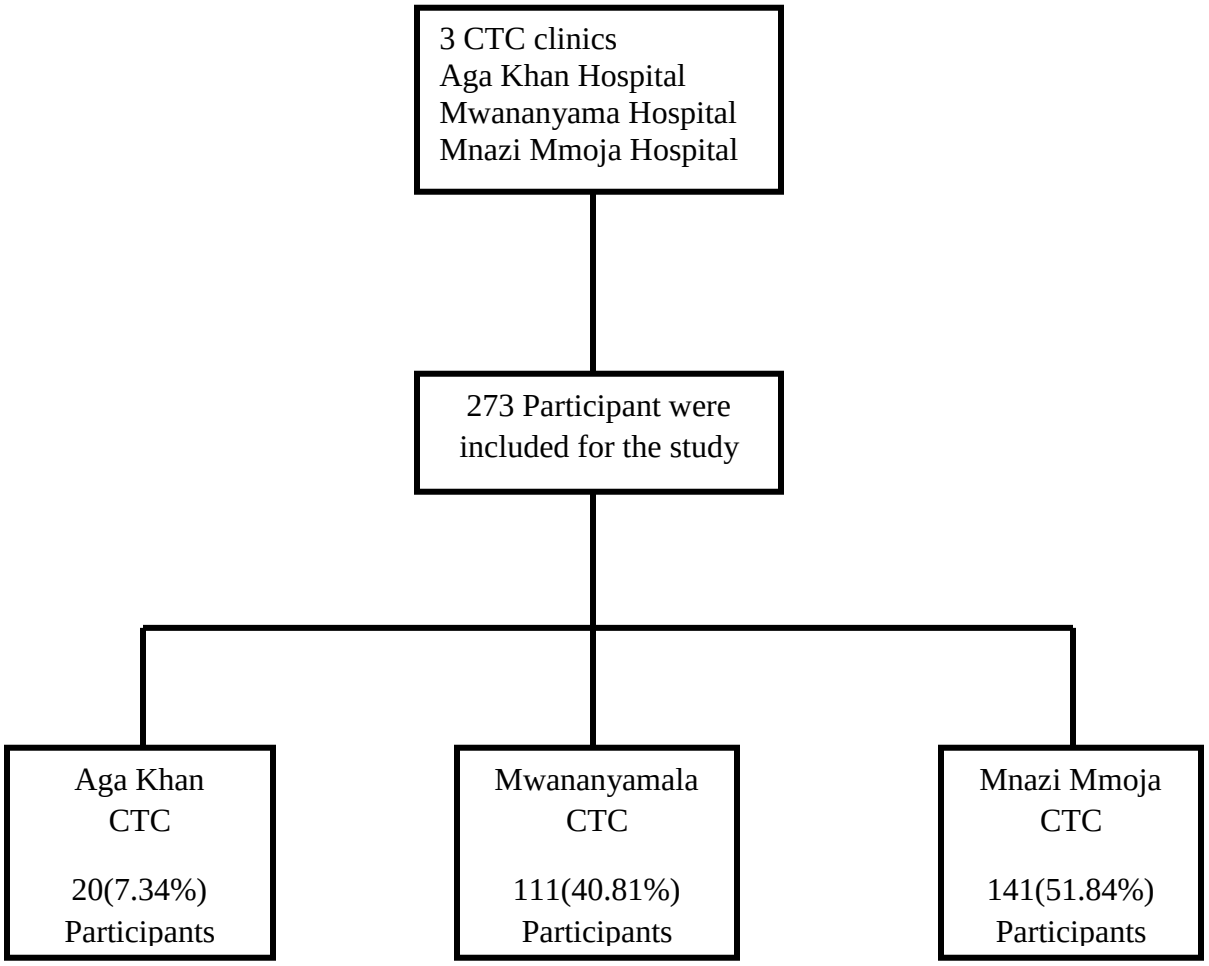


Table 2. Baseline Characteristics of the study participants in the three HIV clinics (n= 273)

Characteristics		ART-Naïve (n=111),40.66%	ART-Experienced Cases(n=162),59.34%	p-value
Age, years mean(SD)= 40.4(9.9)		40.6(10.1)	40.4(9.9)	0.8709
Sex (%)	Male=97(35.5%)	44(39.6%)	53(32.7%)	0.2958
	Female=176(64.5%)	67(60.4%)	109(67.3%)	0.2958
BMI, mean(SD) = 23(4.9)		22.7(4.6)	23.2(5.1)	0.4086
CD4 count, mean(SD)		98(63)		
Viral load(copies/ml),mean,(SD)			179033(761614)	
General Characteristics				
Marital Status no (%)				
Married		123(45.22%)		
Single		92(33.82%)		
Divorced/separated		32(11.76%)		
Widowed		25(9.19%)		
Education Level no (%)				
None		15(5.51%)		
Primary Education		147(54.04%)		
Secondary Education		76(27.94%)		
University/College Education		34(12.5%)		
Nutritional status(BMI)		**Frequency (%)**	**Description**	
Less than 18.5		44(16.24%)	Underweight	
18.5 – 24.9		143(52.77%)	Normal weight	
25 – 29.9		57(21.03%)	Overweight	
30 or more		27(9.96%)	Obese	

The ages of participants ranged from 18 to 74 years (mean, 40.4 years) and there was no significance difference between the ART-naïve and ART – experienced cases. Majority of participants 123(45.22%) were married. More than half of participants had primary education, 5.51% of participants had no formal education and only 12.5% of participants had University/College education.

Nutritional status was assessed through Body mass Index (BMI). The mean BMI of the participants was 23(SD 4.9), and there was no significant difference between the mean BMI of New cases compared to ART- experience cases. Forty four (16.24%) participants were underweight and twenty seven (9.96%) were obese.

The mean CD4 cell count was 98 cells/µL (SD 68 cells/µL) ranging from 7-200 cells/µL in the new cases group of participants. The mean viral load 179,033 copies/mL (SD 761,614 copies/ml) ranging from 1200 – 8900083 copies/ mL.

ART adherence among ART- experienced participants with viral load of ≥ 1000 copies/ml was poor in nearly half of the participants. This is illustrated in figure 4.

Table3. Shows the current ART regiment used by participants in which most patients were on first line treatment and almost 33% were in second line treatment of HIV. Figure 5. Shows common clinical presentations of our participants who were asymptomatic for meningitis but had other symptoms related to other conditions.

Figure 4. ART Adherence among ART- experienced participants in the past six months

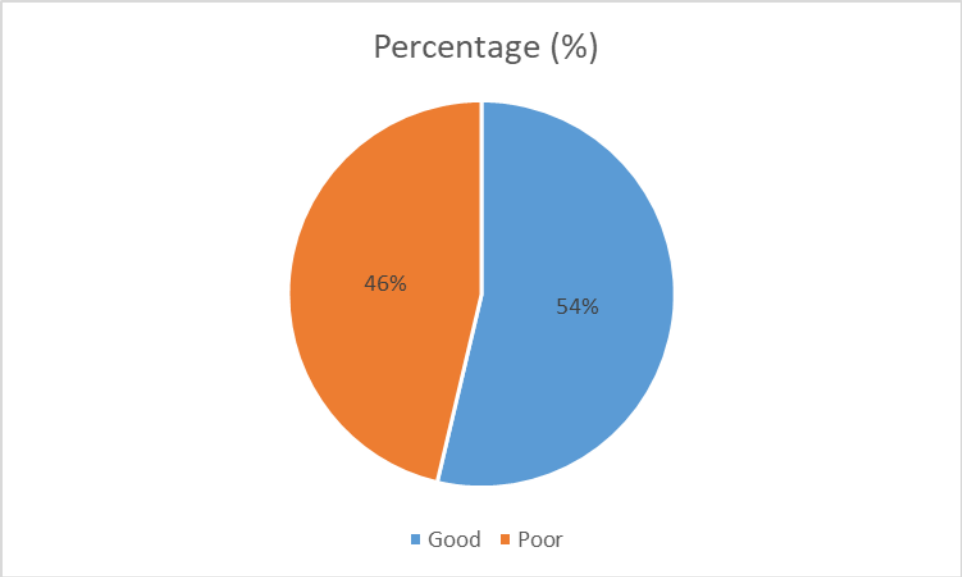
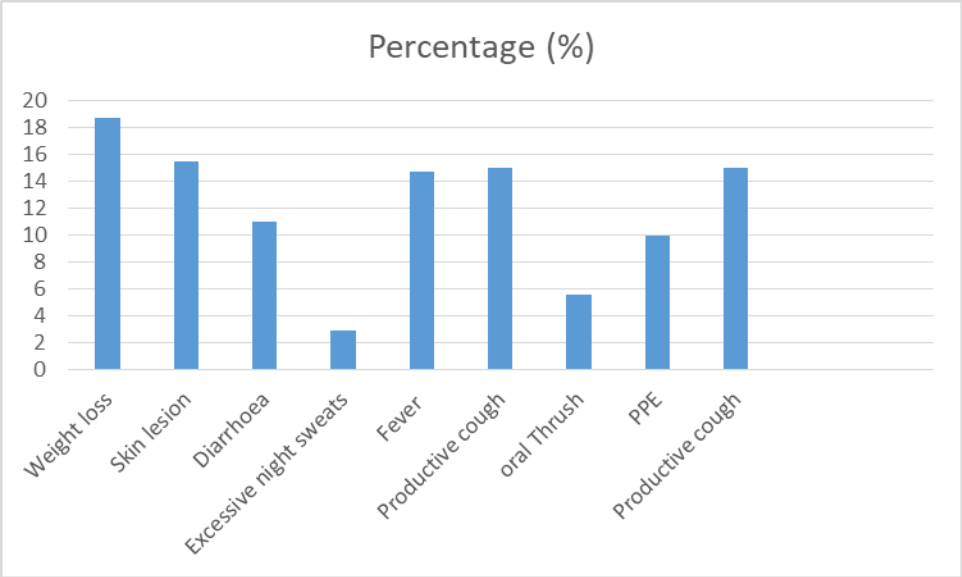


Table 3. Current ART regimen used by participants during the period of the study

Current ART regiment	Frequency	Percent	Cum. Percent
ATV/r Based combination	65	23.81%	23.81%
AZT+3TC+EFV	12	4.40%	28.21%
AZT+3TC+NVP	6	2.20%	30.40%
LPV/r Based combination	28	10.26%	40.66%
TDF+3TC+EFV	156	57.14%	97.80%
TDF+FTC+EFV	6	2.20%	100.00%
Total	273	100%	100%

Figure 5. Other Common clinical presentations in study participants who were asymptomatic for cryptococcal meningitis.



5.1 Prevalence of asymptomatic Cryptococcal antigenemia

Thirteen of the total participants tested positive for cryptococcal antigen (CrAg), giving an estimated overall prevalence of 4.8% (13/273; 95% CI: 2.6 – 8). Nine patients in the new cases group were CrAg positive, giving an estimated prevalence of 8.1% (9/111; 95% CI: 3.8 – 14.8). Four participants in the ART-experienced group were CrAg positive, giving an estimate prevalence in this group to be 2.5% (4/162; 95% CI: 0.7 – 6.2). Fisher exact test was used to compare prevalence between new case and ART-experience group with a p-value (2-tailed) of 0.04 which is statistically significant.

Table 4. Bivariate analysis of factors associated with positive serum Cryptococcal antigen (CrAg)

Variable		CrAg Pos+ (%)	CrAg Neg- (%)	OR	95% CI	p- value
Symptoms						
Fever	**YES**	6 (46.2%)	34 (13.1%)	5.7	1.8 – 17.9	0.003
	NO	7 (53.8%)	226 (86.9%)			
Headache	**YES**	3 (28.1%)	6 (2.3%)	12.7	2.7 – 58.4	0.006
	NO	10 (76.9%)	254 (97.7%)			
Productive Cough YES		1 (7.7%)	40 (15.4%)	0.4	0.05 – 3.6	0.69
NO		12 (92.3%)	220 (84.8%)			
Weight Loss	**YES**	8 (61.5%)	43 (16.5%)	8.1	2.5 - 25.8	0.0005
	NO	5 (38.5%)	217 (83.5%)			
Diarrhea	**YES**	6 (46.2%)	24 (9.3%)	8.4	2.6 – 27.1	0.0002
	NO	7 (53.8%)	236 (90.7%)			
BMI						
≤18		3 (23.1%)	41 (15.9%)	0.5	0.1 – 1.9	0.39
>18		10 (76.9%)	217 (84.1%)			
Signs						
Oral thrush	**YES**	2 (15.4%)	13 (5%)	3.5	0.6 – 17.2	0.15
	NO	11 (84.6%)	247 (95%)			
skin lesions	**YES**	3 (23.1%)	38 (15.1%)	0.6	0.2 – 2.2	0.43
CD4 count cells/µL						
0 -100		8 (72.3%)	54 (48.7%)	0.4	0.1 – 1.4	0.21
101 - 200		3 (27.3%)	57 (52.3%)			
Recent Viral Load (Copies/ml)						
1000 - 50000		2(33.4%)	109 (67.5=7%)	4.2	0.7 – 23.6	0.09
≥50000		4 (66.6%)	52 (32.3%)			
ART Adherence in the past 6 months						
Poor		3 (75%)	72 (45.6%)	0.2	0.02 – 2.7	0.33
Good		1 (25%)	86 (54.4%)			
History of hospital admission in the past 12 months						
YES		8 (61.5%)	28 (10.8%)	13.2	4.0 – 43.1	0.00003
NO		5 (38.5%)	231 (89.2)			

5.2 Analysis of associated factors for asymptomatic cryptococcal Antigenemia

Illustrated in table 4. Several factors were not statistically associated with asymptomatic cryptococcal antigenemia such as BMI, Oral thrush, presence of skin lesions, productive cough and poor adherence in the past 6 months. However, having history of fever in the past two week had odds ratio 5.7 (1.8 – 17.9, $p<0.05$), headache in the past 2 weeks had an odds ratio 12.7 (2.7 – 58.4, $p<0.05$), having history of weight loss in the past one month had an Odds ratio 8.1 (2.5 - 25.8, $p<0.05$), history of diarrhea in the past two weeks odds ratio 8.4 (2.6 – 27.1, $p<0.05$) and history of hospital admission 13.2 (4.0 – 43.1, $p<0.05$) in the past twelve months was statistically associated with asymptomatic cryptococcal antigenemia.

Illustrated in table 5. Logistic regression analysis was done for significant factors that were significantly associated with ACA on univariate analysis. History of headache in the past two weeks and history of hospital admission in the past were found to statistically predict asymptomatic cryptococcal antigenemia.

Table 5. Multiple Logistic Regression Analysis of factors associated with asymptomatic cryptococcal Antigenemia

Variable	OR	95% CI	p-value
Fever	2.7	0.6 – 11.8	0.16
History of hospital admission in the past 12 months	6.6	1.7 – 25.2	0.006
Weight loss	3.7	0.9 - 15.6	0.06
Headache	11.1	1.2 - 100.9	0.03
Diarrhea	3.9	0.9 – 16.5	0.05

5.3 Outcome of Asymptomatic Cryptococcal Antigenemia at 12 weeks

Overall Forty five participants were are followed up for three months from date of enrolment, For every one Cryptococcal antigen positive two Cryptococcal antigen negative participant were followed up through their mobile phone or mobile phone of their relative. Six patients were added to account for loss of follow-up. Because of short duration not all of the participants had completed three month follow-up at time of analysis. All CrAg positive patients completed three months of follow-up, a subset of CrAg negative were selected using simple random technique to make a ratio of CrAg positive to CrAg negative 1:2. Three outcomes were assessed development of symptoms of meningitis (at least two symptoms of meningitis), hospital admission and Mortality.

Four (33.3%) participants out of thirteen who were CrAg positive in the beginning of the study developed symptoms of meningitis (Table 6). Four out of thirteen patients in the CrAg positive participants were admitted in the hospital and there was one death. The likelihood of bad outcome was statistically higher (69%, $p<0.05$) in participants who had CrAg positive test compared to those who had negative CrAg test.

Table 6. Outcomes of Asymptomatic cryptococcal antigenemia at 12 weeks of follow up among patients recruited in the study, Dar es salaam, Tanzania 2019

Outcomes	CrAg Status		P - value
	Positive (n=13)	Negative(n=30)	
Development of symptoms of meningitis (%)	4 (33.3%)	1 (3.3%)	-
Hospital Admission (%)	4 (33.3%)	1 (3.3%)	-
Deaths (%)	1 (8.3%)	0	-
Loss of follow up (%)	1 (7.7%)	3 (9.4%)	
Likelihood of Bad outcome (%)	9 (69%)	2 (4.7%)	0.00001

6.0 DISCUSSION

This is the first study in Tanzania to estimate the prevalence of asymptomatic cryptococcal antigenemia in both ART-naïve and ART experienced patients in three HIV clinics in Dar es salaam city. In our study the overall prevalence of asymptomatic cryptococcal antigenemia among ART-naïve and ART-experienced participants was 4.8%.This prevalence was lower than in two previous studies in Ethiopia by *beyene et al* and *alemu et al* 10.2% and 8.4% respectively (34,35). This difference could be due to two reasons; one is geographical variations cryptococcal antigenemia which has been established in literature and that both these studies did not exclude patients with symptoms of meningitis.

Viral load of more than 1000 copies/ml was used as a criteria to include ART-experienced cases where by CrAg prevalence in this group was 2.5% statistically less than ART-naïve in which prevalence was 8.1% (p=0.04). This difference was similar to the findings by *beyene et al* where majority of patients with CrAg positive were ART- naïve (29). Although another study by *hailu et al* did not find any significant difference in proportion between ART-naïve and ART-experienced patients(34).

The prevalence of 8.1% in ART- naïve patients is similar to other large studies in South Africa by *Jarvis et al* where the prevalence of cryptococcal antigenemia was 7% and Indonesia by *Ganiem et al* where the prevalence was 7.1% (37). In comparison to a study done in two countries by Mfinanga et al in 2015; urban Dar es Salaam was one of the city the study was done and proportion of cryptococcal antigenemia among ART-naïve patients was 5% in Dar es salaam but in this study Latex agglutination method was used to screen for cryptococcal antigenemia in comparison to Lateral flow Assay(LFA) method which was used this study (44). The sensitivity and specificity of CrAg-LFA tests is higher compared to Latex agglutination method, it is simple to use with short turn around time and doesn't require preheating of the sample (31).

The present results have demonstrated that they are asymptomatic cryptococcal antigenemia cases in ART- experienced patients but it is probably not cost effective to screen every one with viral >1000 copies/ml. WHO justify screening if the prevalence of CrAg is >3%, so we need to be more selective in screening ART- experienced patients with high index of suspicion.

The present study determined that history of hospital admission in the past twelve months was associated with cryptococcal antigenemia. This finding was similar to a study *hailu et al* in a recently published study in 2018 (34). History of hospitalization informs clinical deterioration which can be due to treatment failure that may result to emergence of opportunistic infections such as cryptococcus.

There were a couple of symptoms that were associated with cryptococcal antigenemia in univariate analysis but only history of recent headache in the past one week showed significant association with cryptococcal antigenemia. Although headache is one of the symptom of meningitis but on its own its less specific, in a study that was done in Uganda by *Liechy et al* having at least two of more symptoms/signs had more specificity than having one symptoms (39).

This study found that low BMI was not associated with ACA unlike two other studies, one study showed significant association between CrAg antigenemia and having a BMI of less than 18 $Kg/m^{2.}$ (39). Another study done by *oyella et al* found that cryptococcal antigenemia was associated with having BMI of less than 15.4 K/m^2 (31). Our study was done in urban setting compared to a study done by *Liechty et al* which was done in rural Uganda. This present study showed no association between low BMI and ACA, although these two studies were done in different populations another reason could be better nutrition status of urban population in comparison to rural population and this could have affected BMI in our study participants who reside in urban setting (45). More than half of our patient had used ART for more than six months this improves nutritional status in comparison to previous studies that were done in ART-naïve patients only.

In the present study, the proportion of CrAg positive in ART-naïve was no difference when comparing CD4 count between 0-100 and 101-200 cell/ml. This finding is similar to two study one done in Namibia and one done on northern Tanzania. This shows there is a benefits to extend the cutoff point of CrAg screening among ART-naïve patients, however this results should be interpreted with caution due to limitation in sample size and small recruitment of ART- naïve participants in our study. Several other larger studies have demonstrated that CrAg positivity is more common in CD4 count <100 cells/ml.

In the present study there was one death at 3 months but probability of bad event (development of meningitis, hospitalization and death) was statistically higher in CrAg positive patients than CrAg negative patients. Previous studies looked at short term outcomes of cryptococcal antigenemia in ART naïve patients, both were retrospective studies, one done in northern Tanzania there was no difference in mortality between CrAg negative and positive patients at 6 months (12). While the one in Uganda showed 23% mortality at 3 months (39).

Although participants in our study with positive cryptococcal antigenemia were started on treatment there was still poor outcome at 12^{th} week. This could be due to several reasons including adequacy and efficacy of treatment, severity of infection or combination of other diseases.

This present study has observed that the ART- adherence among ART-experienced patients with viral load of >1000 copies/mL was very poor which is usually evaluated in every clinic visit and reported on clinic documents. In ART- experienced patients with viral load of 1000 copies/mL 43.6% were observed to have poor adherence. These findings were similar to another study done in Northern Tanzania by *semvua et al* where the non-adherence was 42% among ART users (46). This could lead to drug resistance and treatment failure that can predispose clinical deterioration of patients with emergence of opportunistic infections such as cryptococcus infection.

The screening test used in this study was simple to use and the participants got their results immediately after interview. The cost of the screening test was relatively inexpensive comparing to other methods, it costed about 1 USD per test and reported to have high sensitivity and specificity in previous studies. It is therefore a practical tool to use in a clinic setting and can be easy in cooperated to the routine care of HIV patients in CTC clinics.

6.1 CONCLUSION

The prevalence of asymptomatic cryptococcal antigenemia in both ART-naive with CD4 count of ≤ 200 cells/µL and ART-experienced with viral load ≥1000 copies/ml was 4.8% in three HIV clinics in Dar es salaam. The prevalence of asymptomatic cryptococcal antigenemia in ART experienced cases for more than 6 months is low compared to new cases of HIV with CD4 count less than 200 cells/µL. There is evidence of extending the spectrum of screening for ACA at higher CD4-count than 100 cells/µL and ART-naïve patients with history of hospitalization in the past twelve months and clinical deterioration. Having a CrAg positive was a predictor of bad outcome at three months with risk of developing symptoms of meningitis, being hospitalized and death. Screening of a subgroup of ART experienced patients with risk factors may reduce mortality associated with cryptococcal meningitis in ART experienced patients.

6.2 RECOMMENDATIONS AND LIMITATIONS

According to the findings this study recommend for extension of cut of CD4 count of less 200 cells/µL to be used for screening of ART naive patients HIV patients. This study also shows there is evidence of CrAg screening of a subgroup of ART-experienced patients there for we recommend a simple algorithm illustrated on figure 6 to be used to screen cryptococcal antigenemia in ART- experienced patient in ambulatory care. This algorithm takes into account index of suspicious of cryptococcal infection depending on several factors determined in our study.

This study had several limitations; the use of recent laboratory values like CD4 count and viral load this were not actual values at the time when screening was done. But this study represents most of our HIV clinics in Tanzania were it takes time to get the results of these tests and in some occasions the machines for testing CD4 counts and viral load can be down for a period of time.

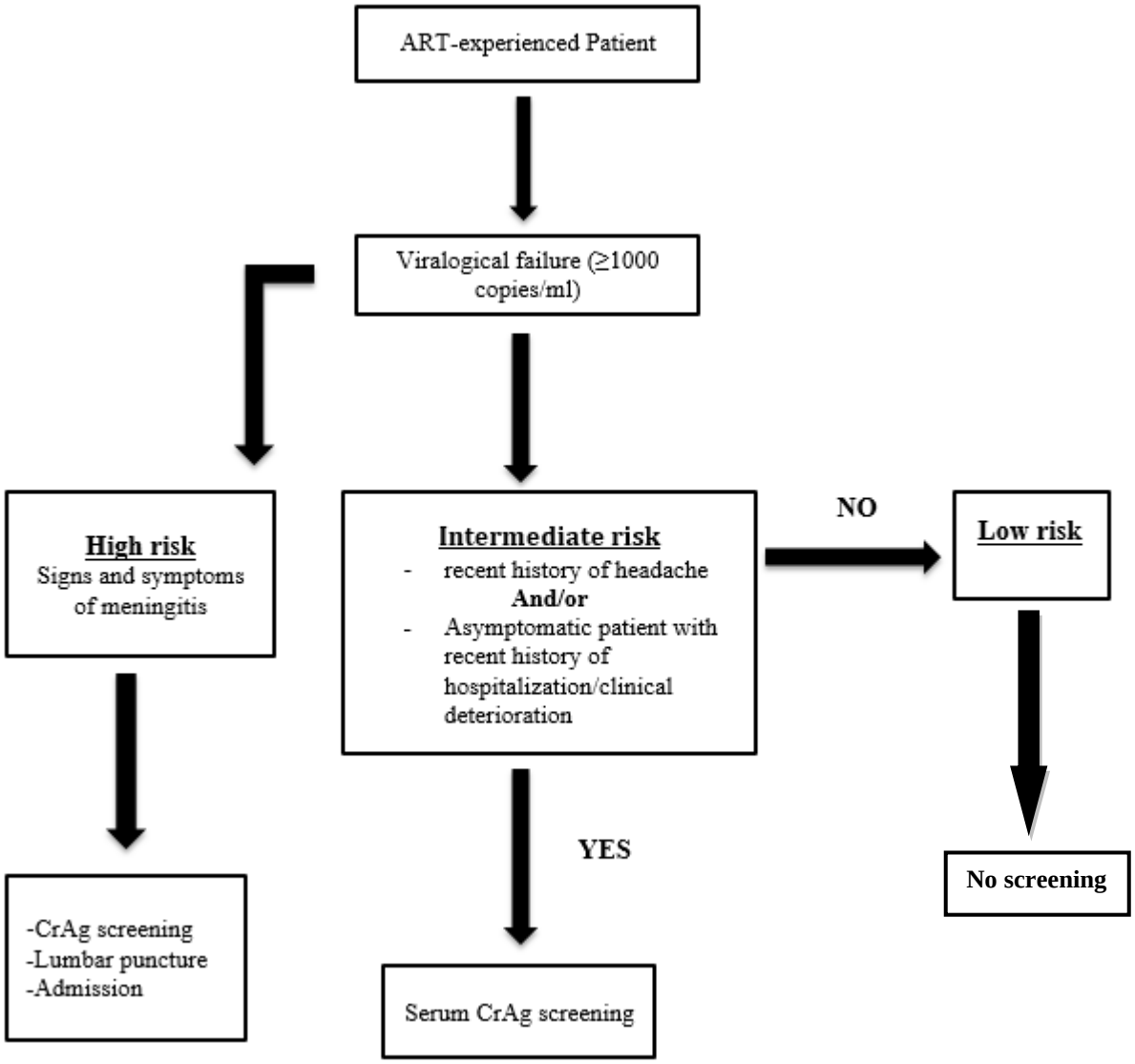
This study could not follow up all the CrAg negative patient at 12 weeks due to time limitations of a master's project. Follow up at three months was done through mobile phones this may have affected

quality of data because there was no face to face contact with the participants. But most study participants and their next of kin were very cooperative and open to share information through a phone call.

This study was not powered enough to determine if there is a difference in outcomes between ART-naïve and ART-experienced with cryptococcal antigenemia at 12th week; therefore this study recommend further studies on that area.

This is a relative small study done in three HIV clinics in one city and might not reflect the country, we recommend further larger studies in this area especially in evaluation of utility of screening of cryptococcal infection in ART- experienced patients with viralogical failure.

Figure 6. Proposed algorithm for Cryptococcal antigenemia screening in ART-experienced patients



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APPENDICES

Appendix 1. Case report form (CRF)

AGA KHAN UNIVERSITY

POSTGRADUATE MEDICAL EDUCATION

Dar es salaam, Tanzania

Case Report Form

PREVALENCE AND OUTCOME OF ASYMPTOMATIC CRYPTOCOCCAL ANTIGENEMIA IN HIV PATIENTS (ART naïve and ART experience) PATIENTS IN DAR ES SALAAM (ACRAD Study)

Chief/Principal Investigator:

Subject ID number

☐☐☐

Subject Initials

☐☐☐

Center ID

☐☐☐

Date of enrolment:

Subject ID number

1.Patients Demographics and physical characteristics

Age (years):

Gender: ☐ Female
☐ Male

Marital status: ☐ Single ☐ Married ☐ Divorced/Separated ☐ Widowed

Occupation:……

Education level: ☐ Primary education ☐ Secondary education ☐ Higher learning

The number of years of education:

Weight (Kg):

Height (cm):

2.Meningitis symptoms screen

I.Any history of fever for the past one week?

☐YES ☐NO

II.Does the patient have a severe headache or history of a severe headache in the past one week?

☐YES ☐NO

III.Does the patient have neck stiffness?
☐YES ☐NO

IV.Does the patient have a new onset of altered mentation/confusion?
☐YES ☐NO

V.Does the patient have new onset of seizures?
☐YES ☐NO

VI.Does the patient have new onset of visual impairment?
☐YES ☐NO

3. Other symptoms/signs screen

I.Is there any recent (in the past two weeks) history of diarrhea?
☐YES ☐NO

II.Is there any recent (in the past one month) history of subjective weight loss?
☐YES ☐NO

III.Is there any recent history of a productive cough?
☐YES ☐NO

IV.Is there any recent history of excessive night sweats?
☐YES ☐NO

V.Is there any history of painful swallowing?
☐YES ☐NO

VI.Any Obvious skin lesion:☐ YES ☐ NO;

If yes are the lesion:
Papules ☐YES ☐ NO
Plaques ☐YES ☐NO
Purpura ☐YES ☐NO
Ulcers ☐YES ☐NO

OTHERS: Please describe ……..

VI. Oral candidiasis (please examine if there is a whitish curd like lesions in the oral cavity:
☐YES ☐NO

Subject ID number

☐☐☐

4.HIV/AIDS history

Variables	At diagnosis	Recent	% Change
CD4 Count			
Viral Load			
WHO Clinical Stage			

5.ARV treatment

I. Duration of ARV treatment (in months): …….

II. Current ARV regiment (list the drug names):
……

III. List any previous regiment used in the past;
1st …….

2nd ……

3rd ……

6.Past Medical History

Comorbid:
I. Does the patient have any chronic medical problems? ☐YES ☐NO

II. Diabetes mellitus ☐YES ☐NO

III. Cancer ☐YES ☐NO

IV.Organ transplant ☐YES ☐NO

V.OTHERS list them……

Subject ID number

Medications:

I. Does the patient use any other medications apart from ARV? ☐YES ☐NO
If YES list them; ……

The number of hospitalization in the last year:
I. Was patient admitted to the hospital in the past 12 months? ☐YES ☐NO

If YES how many times was he admitted? .....

Why was he admitted? .....

Appendix 2. Informed Consent form (Swahili version)

AGA KHAN UNIVERSITY
Internal Medicine Department, Faculty of Medicine (PGME)

FOMU YA KUOMBA RIDHAA

MTAFITI: DR. MANDELA CHARLES MAKAKALA

(MD, Cert. International Public health and HIV/AIDS)

Hii ni Fomu ya ridhaa ni kwaajili ya wagonjwa ambao wanahudhuria kliniki za CTC jijini Dar es salaam. Sisi tunawakaribisha kushiriki katika utafiti juu ya maambukizi cryptococcal. Jina la utafiti wetu ni Ukubwa wa maambukizi na matokeo ya dalili cryptocoocal antigenemia kwa wagonjwa walioambukizwa VVU katika mkoa wa Dar es salaam. Fomu hii ina sehemu mbili ; Maelezo juu ya utafiti huu na sehemu ya kuridhia kushiriki katika utafiti huu.

SEHEMU I: Maelezo juu ya utafiti

Mimi ni Dr. Mandela Makakala, Mwanafunzi wa udaktari bingwa magonjwa ya ndani katika chuo kikuu cha Tiba Aga Khan Kilicho jiji la Dar es salaam. Tunafanya utafiti juu ya maambukizi cryptococcal ambayo kwa kawaida huathiri wagonjwa wenye upungufu wa kinga mwilini hasa wale wanaoishi na maambukizi ya VVU. Nitakwenda kukupa maelezo kuhusu utafiti huu na kisha kukukaribisha kushiriki katika utafiti huu. Si lazima kuamua leo iwapo unataka kushiriki katika utafiti. Kabla ya kuamua, unaweza kuzungumza na mtu yeyote mwingine kabla ya kufanya maamuzi.

Madhumuni ya utafiti

Utafiti huu unalenga kuchunguza maambukizi cryptococcal katika watu wanaoishi na VVU. Hii ni maambukizi ya kawaida sana hasa katika watu wanaoishi na Maambukizi ya VVU kwa sababu ya kinga kuwa chini .Huu ni ugonjwa wa fangus unaosababisha homa ya uti wa mgongo. Maambukizi haya yasipotibiwa mapema unaathiri ubongo na katika hali nyingi husababisha kifo, maambukizi ya ugonjwa huu ni sababu kubwa ya vifo katika watu wanaoishi na maambukizi ya VVU. Kama ugonjwa ukigunduliwa katika hatua za mwanzo unaweza kutibiwa ili kuzuia madhara. Hivyo utafiti huu unalenga kuangalia idadi wagonjwa HIV na wenye maambulizi ya Cryptococci na vitu vinavyochangia maabukizi hayo.

Pale ambapo utakubali kushiriki katika utafiti huu taarifa zako zitakuwa siri kwa watafiti tu. Utapewa namba kwa ajili ya kutambua, jina lako halitatumika katika hati za tafiti. Sisi itahitaji ndogo ya damu sampuli ya kuangalia ya maambukizi cryptococci. Kama kipimo kitaonyesha unamaambukizi ya cryptococci tutakuongoza kwa daktari wako kwa ajili ya matibabu, tutakuwa kufuatilia hali yako kwa muda wa miezi mitatu katika kliniki. Kama kipimo chako kitakuwa hasi utapewa taarifa na kupewa ushauri juu ya dalili za ugonjwa huu.

Ili kufanya kipimo tutatoa damu kidogo kwa sindano ambayo itafanyiwa kipimo cha Cryptococci. Kuna weza kuwa na maumivu kidogo wakati wa kutoa damu,lakini ni maumivu ya sindani ya kawaida kama unapofanya vipimo vingine.

Muda wa utafiti

Muda wa utafiti utakuwa ni miezi mitatu, utaonana na mtafiti mara mbili yani mwanzoni mwa utafiti na baada ya miezi mitatu.

Umuhimu wa utafiti huu

Ugonjwa huu ni ugonjwa hatari sana ambao unaweza kusababisha kifo, unapogununduliwa mapema unaweza kupata matiba ya dawa za kunjwa na kupona kabisa. Kushiriki katika utafiti huu utakupa nafasi ya kupima na kupata elimu juu ya dalili za ugonjwa huu. Utafiti huu hauta gharamia dawa za matibabu endapo utagunduliwa na maambukizi haya.

Haki ya kutoshiriki/Kuamua kujitoa kwenje utafiti

Una haki yakutoshiriki katika utafiti huu usiporidhia na pia una haki ya kujitoa katika hatua yeyote ya utafiti huu.

SEHEMU YA PILI: Cheti cha kuomba ridhaa

Nimesoma na nimeelezewa kuhusu utafiti huu, na kwa utashi wangu nimeamua kushiriki katika utafiti huu.

Jina la Mshiriki _____ **Kama hawezi kuandika; Weka alama ya kidole**

Saini ya mshiriki_____

Tarehe _____

siku/mwezi/mwaka

Nina thibitisha kwamba mshiriki katika utafiti huu amepewa taarifa zote kuhusu utafiti huu, amepata nafasi ya kuuliza maswali na kujibiwa na amekubali kwa hiari na utashi wake kushiriki katika utafiti huu bila kulazimishwa.

Jina la mtafiti _____

Saini ya mtafiti_____

Tarehe _____

Siku/mwezi/mwaka

Appendix 3. Informed consent form (English version)

AGA KHAN UNIVERSITY
Internal Medicine Department, Faculty of Medicine (PGME)
INFORMED CONSENT FORM
RESEARCHER: DR. MANDELA CHARLES MAKAKALA
(MD, Cert. International Public health and HIV/AIDS)

This Informed Consent Form is for patients who attend CTC clinics in Dar es salaam, and who we are inviting to participate in research on cryptococcal infection. The title of our research project is Prevalence and outcome of asymptomatic cryptococcal antigenemia in HIV infected patients in Dar es salaam. This informed consent has two parts Information Sheet and Certificate of Consent. PART I: Information Sheet

Introduction

I am Dr. Mandela Makakala; Internal Medicine resident at the Aga Khan University Dar es salaam campus. We are doing research on cryptococcal infection which commonly affects patients will low immunity especially those living with HIV infection. I am going to give you information then invite you to participate in this research. You do not have to decide today whether or not you will participate in the research. Before you decide, you can talk to anyone you feel comfortable with about the research.

Purpose of the research

This research aims at investigating cryptococcal infection in people living with HIV. This is a very common infection particularly in people living with HIV Infection because of low immunity. If the infection is not treated early it affects the brain and in most cases it causes death, this infection is the leading cause of death in people living with HIV infection. If caught in early stages it may be treated to prevent complications. So this study aims at how many HIV patients have an early cryptococcal infection, what risk factors that caused the infection and how the infection will affect them.

Procedures and Protocol

If you agree to participate in our study clinical and relevant information will be obtained from. This information will be confidential and will not be shared with other people. You will be given a number for identification and your name will not be in the documents. We will require a small blood sample to check for cryptococcal infection. If the test is positive for the infection we will refer you to your doctor for treatment, we will follow up your condition for three months in the clinic. If your test is negative you will be informed about the result. You will be educated on symptoms of the disease so that you can seek management early if you acquire the infection.

We will take blood from your arm using a syringe and needle only once during the period of study. This might inflict little injection pain. The blood sample will be destroyed by the end of the study.

Duration

You will participate in the study for three months, there are going to be two contacts with the researcher, at the beginning of the study and at the end of three months.

Benefits

This study will offer you a chance to screen for this severe infection at early stages and if positive you will be referred to your doctor for treatment. You will also receive information about the disease relevant to prevention and management of the disease.

Right to Refuse or Withdraw

You do not have to take part in this research if you do not wish to do so and refusing to participate will not affect your treatment at this clinic in any way. You will still have all the benefits that you would otherwise have at this clinic. You may stop participating in the research at any time that you wish without losing any of your rights as a patient here. Your treatment at this clinic will not be affected in any way.

PART II: Certificate of Consent

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Name of Participant_____ If illiterate; Thumb print of participant

Signature of Participant _____

Date _____

Day/month/year

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Name of Researcher/person taking the consent_____

Signature of Researcher /person taking the consent_____

Date _____

Day/month/year

Appendix 4.Blood sample report form

AGA KHAN UNIVERSITY
POSTGRADUATE MEDICAL EDUCATION
Dar es salaam, Tanzania

BLOOD SAMPLE REPORT FORM

Subject ID number

☐☐☐

Subject Initials

☐☐☐

Center ID

☐☐☐

Procedure

A minimum of 2cc of blood will be collected from a vein with a 5 cc needle. Blood will be collected to a plain vacutainer and LFA CrAg kit will be used to test the sample. If the result is a positive quantification of CrAg Titers will be done. Positive patients will be informed their results referred to their doctor for preempting treatment of cryptococcal antigenemia.

The result of serum cryptococcal antigen.

Please fill the test results of serum cryptococcal antigen

LFA CrAg TEST RESULTS; POSITIVE ☐

NEGATIVE ☐