CRYPTOCOCCAL MENINGITIS IN HOSPITALIZED HIV PATIENTS AT THE FEVERS' UNIT, KORLE-BU TEACHING HOSPITAL, ACCRA

By

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DECLARATION

The work in this thesis is authentic and original and was carried out at the Department of Microbiology, School of Biomedical and Allied Health Sciences and the Central Laboratory, Biochemistry Department, Korle-Bu Teaching Hospital (KBTH), by me and supervised by the supervisors below. Work from other authors that were used were duly acknowledged in the text or by reference cited. This work has not been submitted at any institution wholly or partially for the award of any degree.

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This work is dedicated to God and my family.

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ABSTRACT

Introduction: Cryptococcal meningitis (CM) is the leading form of meningitis in HIV/AIDS patients and causes significant morbidity and mortality, even in the advent of accessibility of antiretroviral therapy. Globally, the burden of CM is estimated to be at 9.6 million cases and results in about 6.2 million deaths within three months of infection.

General aim: The aim of the study was to determine the prevalence of Cryptococcal meningitis in hospitalized HIV-infected patients at the Fevers' Unit, Korle-Bu Teaching Hospital.

Method: A cross sectional study on fifty-three consecutive hospitalized HIV patients with clinical presentations suggestive of meningitis at the Fevers' Unit of the Korle-Bu Teaching Hospital was undertaken within a period of 12 months. Cerebrospinal fluid (CSF) Cryptococcal Antigen Lateral Flow Assay (CrAgLFA), culture and microscopy (India ink, Gram stain) were performed on CSF samples taken by lumbar puncture. Sensitivity and specificity analysis of serology, India ink and Gram stain microscopy were performed using culture as gold standard. CSF White Blood Cell (WBC) count, CSF biochemistry and viral load were also done. Patient's folders were also audited and analyzed.

Results: A total of 53 CSF specimens were collected for the study. Out of 53 subjects, 28 (53%) were males and 25 (47%) were females with mean age of 40.7 years. Headache, fever, confusion-neurosymptoms, oral thrush, meningismus and tachycardia were major clinical presentations shown by the subjects. One participant was confirmed positive for cryptococcal meningitis by CrAgLFA, India ink and Gram stain but not culture giving a prevalence of 1.9% among the study subjects. The sensitivity and specificity of CrAg and microscopy against culture were 0% and 98.1% respectively. The confirmed CM patient showed headache, stiff neck and unstable gait. Twelve (22.6%) had CSF WBC counts above

20 cells/mm^3 with lymphocytic predominance. CD4 count below 100 cells/µl were seen in 20 (37.7%) of the subjects and 43.3% of them had viral loads above 1.0×10^5 RNA copies/ml. Eleven (20.8%) were on Anti-retroviral therapy (ART) with 46 (86.8%) in WHO stage IV of HIV. Nine (17.0%) of subjects had CSF glucose below 2.5 mmol/L, 31 (58.5%) had positive CSF globulin and 32 (60.4%) had CSF total protein greater than 0.45 g/L. Forty-three (81.1%) had haemoglobin level below 12.0 g/dl with 19 (35.9%) having abnormal Total WBC. Fourteen (26.4%) presented with abnormal platelet count while 9 (17.0%) had abnormal Random blood glucose. Thirty (56.6%) and 5 (9.4%) were put on oral fluconazole and IV fluconazole whiles others were put on antibiotics, antimalarials and analgesic. The CM confirmed patient had haemoglobin level of 10.5 g/dl, WBC of 6.8×10^9/L, Platelet of 129×10^9/L, RBS of 8.0 mmol/L, CSF glucose of 2.8 mmol/L, CSF/Serum glucose ratio of 0.35, CSF protein of 0.47 g/L, Globulin was negative, CSF WBC count of 235 cells/mm^3 with lymphocytic predominance, CD4 count of 182 cells/µl, viral load of 482 RNA copies/ml, was ART compliant, Glasgow coma score of 15/15, WHO stage IV and was put on oral fluconazole. The mortality rate for the study was 39.6%.

Conclusion: There was low prevalence of cryptococcal meningitis which suggests that CM may not necessarily be the leading form of meningitis among HIV/AIDS subjects at the Fevers' Unit, Korle-Bu Teaching Hospital. The sensitivities and specificities of serology, India ink and Gram stain were 0% and 98.1% each respectively. Culture should be coupled with other diagnostic parameters (Serology, India ink and Gram stain) for reliable CM diagnosis.

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LIST OF ABBREVIATIONS

% - Percentage

˚C - Degree Celsius

µm - Micrometer

AFLP - Amplified Fragment Length Polymorphisms

AIDS - Acquired Immunodeficiency Syndrome

ART - Anti-Retroviral Therapy

BHI - Brain Heart Infusion

C. gattii - *Cryptococcus gattii*

C. neoformans - *Cryptococcus neoformans*

cells/µl - Cells per microliter

cells/mm^3 - Cells per cubic millimetre

cells/L - Cells per litre

CD4 - Cluster of Differentiation 4

CM - Cryptococcal meningitis

CNS - Central Nervous System

CO_2 - Carbon dioxide

CrAg - Cryptococcal antigen

CrAgLFA - Cryptococcal Antigen Lateral Flow Assay

CSF - Cerebrospinal fluid

EDTA - Ethylene diamine tetra acetic acid

EIA - Enzyme Immunoassay

ESR - Erythrocyte Sedimentation Rate

FU - Fevers' Unit

g - Gram

g/dl - Gram per decilitre

g/L - Gram per litre

HAART - Highly Active Antiretroviral Therapy

Hb - Haemoglobin

HIV - Human Immunodeficiency Virus

ID - Identification

IV - Intravenous

KBTH - Korle-Bu Teaching hospital

L - Litre

LA - Latex Agglutination

LP - Lumbar puncture

ml - Millilitre

MLST - Multi-locus Sequence Typing

mm^3 - Cubic millimetre

mm/µl - Millimetre per microliter

mm/hr - Millimetre per hour

mmol/L - Millimole per litre

nm - Nanometer

NMINR - Noguchi Memorial Institute for Medical Research

PCR - Polymerase Chain Reaction

PI - Principal investigator

PMNs - Polymorphonuclear neutrophils

RBC - Red Blood Cell

RBS - Random Blood Sugar

RFLP - Restriction Fragment Length Polymorphism

RLS - Resource Limited Setting

RNA – Ribonucleic acid

RPM - Revolution per minute

SBAHS - School of Biomedical and Allied Health Sciences

SD – Standard deviation

SDA - Sabouraud Dextrose Agar

SOP - Standard Operation Procedure

TCA - Trichloroacetic acid

VG - Variety *gattii*

VN - Variety *neoformans*

WHO - World Health Organization

WBC - White Blood Cell

CHAPTER ONE

INTRODUCTION

1.0 BACKGROUND

Meningitis is defined as the inflammation of the meninges covering the brain and spinal cord (Nester *et al*., 2009). This occurs mostly due to an infection in the cerebrospinal fluid (CSF) (Feldman, 1977; Garges *et al*., 2006). Meningitis can be caused by a dazzling array of etiologic agents such as bacteria, fungi, viruses, parasites and chemicals; however, bacteria are the most common etiologic agents implicated in causing meningitis (Reid and Fallon, 1992; Rotbart, 1999; Katwere *et al*., 2009; Cohen *et al*., 2010). Meningitis can be acute or chronic and the onset of symptoms may be quick or last a month or more, respectively (Anderson *et al*., 1987; Spanos *et al*., 1989).

Certain bacteria are implicated in causing meningitis. However, *Streptococcus pneumoniae*, *Neisseria meningitides* and *Haemophilus influenzae* are the mostly implicated bacterial agents (Maleeha *et al*., 2006; Janocha-litwin and Simon, 2013). Certain anatomical defects predispose a patient to recurrent bacterial meningitis (Lieb *et al*., 1996). These anatomical defects may be congenital or acquired, or by disorders of the immune system (Lieb *et al*., 1996; Brouwer *et al*., 2010). Tuberculous meningitis (TBM) is the type of meningitis that is caused by *Mycobacterium tuberculosis* (Kennedy and Fallon, 1979; Carbonnelle, 2009). TBM is prevalent in immunocompromised patients like Human Immunodeficiency Virus (HIV)/Acquired Immunodeficiency Syndrome (AIDS) patients (Berenguer *et al*., 1992; Carbonnelle, 2009). Viral or aseptic meningitis is caused by certain viruses (Rotbart, 1997; Carbonnelle, 2009). It is generally mild and self-limiting without specific treatment (Kumar, 2005; Ooi *et al*., 2010). Although viral meningitis is life threatening, in the

immunocompetent people, it is rarely fatal, with symptoms lasting for 7 to 10 days after which the patient recovers fully (Chadwick, 2005; Carbonnelle, 2009). Viral meningitis and bacterial meningitis in their early phase present with similar symptoms (Carbonnelle, 2009). Fungal meningitis is a rare but life threatening condition that is common in immunocompromised patients (Garber, 2001; Nester *et al*., 2009). *Cryptococcus* species, a fungus, is the leading cause of meningitis in immunocompromised people (Mitchell and Perfect, 1995; Hakim *et al*., 2000; Park *et al*., 2009). Cryptococcal meningitis (CM) is the term given to meningitis caused by *Cryptococcus* species (Raman Sharma, 2010).

Cryptococcal meningitis is the leading form of meningitis in HIV/AIDS patients and causes significant morbidity and mortality, even in the advent of accessibility of antiretroviral therapy (French *et al*., 2002; Fang *et al*., 2004; Beyrer and Abdool Karim, 2013). Globally, the burden of CM is estimated to be at 9.6 million cases and results in about 6.2 million deaths within three months of infection (Bicanic *et al*., 2007; Park *et al*., 2009; La Hoz and Pappas, 2013). There is high morbidity and mortality relative to CM in southern and Eastern Africa. This can be attributed to the high HIV prevalence, unavailable antifungal therapies and late diagnosis in that geographical locality (Kambugu *et al*., 2008). Furthermore, in South Africa, a study showed a change in the epidemiology of meningitis where cryptococcal meningitis was recorded as the leading cause of community-acquired meningitis with about 20-45% prevalence (Hakim *et al*., 2000). Frimpong and Lartey (1998) recorded no prevalence in 28 HIV patients whiles Owusu *et al*., (2012) recorded about 11.7% of CM prevalence in Kumasi. There has however been a substantial decrease in the incidence of CM in developed countries and this can be attributed to improved access to antiretroviral therapy (ART) and optimal antifungal therapy (Mathiesen *et al*., 2012; Asboe *et al*., 2012; Govender *et al*., 2013).

The causative organism for Cryptococcal meningitis, *Cryptococcus neoformans*, is ubiquitous and saprophytic in nature (Ellis and Pfeiffer 1990; Levitz, 1991; Gugnani *et al*., 2005). Because of its ubiquituousness, it is assumed that exposure to *C. neoformans* is common (Levitz, 1991), however, there was high immunological resistance to cryptococcal infection because its incidence was relatively rare until the start of the Acquired Immunodeficiency Syndrome (AIDS) (Ellis and Pfeiffer 1990; Gugnani *et al*., 2005; Nester *et al*., 2009).

Cryptococcal meningitis can mimic tuberculosis, other mycoses, viral meningoencephalitis or meningeal metastases (Sabetta and Andriole, 1985; Perfect and Casadevall, 2002). Aside an abnormal mental status, nuchal rigidity, abnormal chest radiology and hyponatraemia, tuberculous meningitis may be difficult to distinguish from Cryptococcal meningitis (Sabetta and Andriole, 1985; Perfect and Casadevall, 2002). Other mycoses that may mimic CM are histoplasmosis, coccidiodomycosis, norcadiosis or aspergillosis (Sabetta and Andriole, 1985; Perfect and Casadevall, 2002). Meningeal metastases like lymphoma may also mimic Cryptococcal meningitis (Glass *et al*., 1979).

Symptoms of cryptococcal meningitis in healthy individuals develop very gradual. On the contrary, there is generally faster progression of the disease symptoms in the immunocompromised (Nester *et al*., 2009). Without early diagnosis and treatment, death can occur within two weeks (Nester *et al*., 2009). Symptoms of the disease include headache, unexplained fever, nausea and vomiting, dizziness, neck stiffness, confusion, seizures, abnormal behavior, new-onset psychiatric symptoms, altered level of consciousness, focal neurological signs, diplopia, unexplained blindness, paralysis, and coma (McCarthy *et al*., 2006; Nester *et al*., 2009).

Cryptococcal infection in the Central Nervous System almost always causes abnormalities in the CSF. In a study where forty (40) CM patients were recruited, 64% of the patients had

their opening pressure elevated (Butler *et al*., 1964). There was also low CSF glucose concentration and high CSF protein concentration. The cell counts in CSF were usually abnormal with leukocyte counts exceeding 20/mm^3 and lymphocytes generally outnumbering neutrophils (Schmidt *et al*., 1995). All these parameters may however be misleading in diagnosing CM (Butler *et al*., 1964).

CSF culture is the mainstay diagnostic tool; therefore isolating the organism from the CSF can give a definitive diagnosis of CM. Other classical methods of diagnosis are India ink preparation of the CSF and serologic method (Dismukes *et al*., 1987; Saha *et al*., 2008). Serologic test has also been shown to be a useful diagnostic tool when CSF cultures and India ink preparations are negative (Goodman and Kaufman, 1971; Snow and Dismuke, 1975).

Cryptococcal meningitis is fatal if untreated and because of the infection's proximity to the brain and spinal cord, it is termed medical emergency (Butler *et al*., 1964). Treatment is done by using Ampotericin B and flucytosine combination and azoles like fluconazole, itraconazole and voriconazole (Diamond *et al*., 1998; Larsen *et al*., 2004; Govender *et al*., 2013). Combination therapy produces a higher success rate in treating CM infection (Larsen *et al*., 2004; Perfect *et al*., 2010). Early diagnosis and treatment may go a long way to prevent CM associated morbidities and mortalities. In order to diagnose CM, lumbar puncture (LP) is carried out to obtain CSF for India ink microscopy in Resource limited settings (RLS) (Andama *et al*., 2013). However, the preliminary symptoms of headache and fever are not differential clinical sign and symptom to meningitis so an LP is done when the disease is in its advanced stage (Govender *et al*., 2013).

The present study aimed to ascertain the prevalence of cryptococcal meningitis among HIV patients at the Fevers' Unit, Korle-Bu Teaching Hospital, Ghana.

1.1 PROBLEM STATEMENT

Even in the advent of antiretroviral therapy (ART) accessibility, Cryptococcal meningitis continues to be the leading form of meningitis in HIV/AIDS patients (Antinori *et al*., 2009; Andama *et al*., 2013). It is estimated that about 9.6 million CM cases occur globally which leads to about 6.2 million deaths within three months of infection (La Hoz and Pappas, 2013). Although there is availability of antifungal therapies, the morbidity and mortality rate of CM in Southern and East Africa are very high (Park *et al*., 2009). Adult patients in the Sub-Saharan Region suffering from CM records an alarming case fatality between 35%-65%. On the contrary, the case fatality rate of adult CM patients in the developed countries is between 10%-20% (Andama *et al*., 2013).

Many deaths from CM may be prevented through early diagnosis and treatment, but the rapid serum cryptococcal antigen test is often not available in our hospital settings. Diagnosis of CM is usually done by India ink microscopy of cerebrospinal fluid (CSF) in RLS (Andama *et al*., 2013). India ink microscopy is less sensitive and specific as compared to cryptococcal antigen (CrAg) test (Boulware *et al*., 2014); however, the preliminary symptoms of headache and fever are not differential clinical presentations to CM and meningitis of other aetiologic agents, so LP is often done when the disease is in its advanced stage (Ginsberg, 2004; Govender, 2013).

This study sought to investigate the prevalence of cryptococcal meningitis in hospitalized HIV-infected patients at the Fevers' Unit, KBTH, Accra. In addition, ascertaining the sensitivities and specificities of serology and microscopy using culture as gold standard as well as the use of CSF White Blood Cell (WBC) count, CSF biochemistry and viral loads to confirm CM in HIV-infected patients would enhance CM diagnosis in Ghana.

1.2 JUSTIFICATION

There is scanty information on the prevalence of CM among HIV patients in Ghana. Two findings from Kumasi showed contrasting disease prevalence of zero percent (0%) and 11.7%. The HIV status of these subjects are however unknown. The HIV prevalence in Ghana is about 1% with an annual death of 25,000 (UNAIDS, 2013). These HIV patients are at risk of developing CM.

Ascertaining CM prevalence and in-hospital mortality among HIV/AIDS patients in the largest Teaching Hospital in Ghana will enhance our understanding and improve clinical care. Furthermore, outcome of this study will be used as a tool to convince important stakeholders to invest more in the management of cryptococcal meningitis.

1.3 AIMS AND OBJECTIVES

1.3.1 Aim

The aim of this study was to investigate the prevalence of cryptococcal meningitis in hospitalized HIV-infected patients at the Fevers' Unit, KBTH, Accra.

1.3.2 Specific objectives

- To determine CSF White Blood Cell (WBC) counts, CSF biochemistry, Cluster of differentiation 4 (CD4) count and viral loads of the HIV-infected patients.

- To determine outcome of CM using clinical presentations, CSF White Blood Cell (WBC) count, CSF biochemistry, CD4 count and viral loads in the HIV-infected patients.

- To determine the sensitivities and specificities of serology and microscopy (Gram stain, India ink) using culture as gold standard in confirming CM in the HIV-infected patients.

CHAPTER TWO

LITERATURE REVIEW

2.1 MENINGITIS IN HIV INFECTED PATIENTS

Meningitis is defined as the inflammation of the meninges (Nester *et al*., 2009) and it is a common complication in persons infected with the Human Immunodeficiency Virus (HIV) (Hakim *et al*., 2000; Nester *et al*., 2009).

Meningitis in HIV patients is almost always of infectious origin with two opportunistic organisms causing about 75 percent of it (Kambugu *et al*., 2008). They are *Cryptococcus neoformans* and *Mycobacterium tuberculosis* with *Cryptococcus neoformans* being the most common etiologic agent and systemic fungal agent of meningitis in HIV patients (Kambugu *et al*., 2008). In a cross-sectional study on HIV subjects, Cohen and colleagues confirmed CM and TBM in 43% and 18% of their subjects, respectively (Cohen *et al*., 2010). *Streptococcus pneumoniae*, *Neisseria meningitides*, and *Treponema pallidum*, fungi other than *C. neoformans* (*Aspergillus* spp.), parasites (*Naegleria fowleri, Toxoplasma gondii*) and viruses are other aetiologic agents that can cause meningitis in HIV patients (Katwere *et al*., 2009; Cohen *et al*., 2010). In contrast, meningitis can be a presentation of acute infection of HIV itself (Price, 1996); however, in the advanced stages of HIV infection (WHO defined stages 3 and 4), *Cryptococcus* spp is a common cause of meningitis and death (Kambugu *et al*., 2008; Katwere *et al*., 2009; Desalermos *et al*., 2012).

2.2 PREDICTIVE CLINICAL PRESENTATIONS FOR CRYTPOCOCCAL MENINGITIS IN HIV PATIENTS

Cryptococcal meningitis is mostly associated with HIV patients usually with CD4 count below 100 cells/mm^3 (Dismukes, 1988). In a study where clinical presentations were used as

predictive values for CM in 149 HIV in-patients, patients having CD4 counts below 100 cells/mm^3 were prone to CM with a sensitivity of 93%. On the other hand, coma had the highest specificity (84%) in the absence of CM. A combination of CD4 count below 100 cells/mm^3 and coma gave the highest positive predictive values (58%) with a positive likelihood ratio of 3.8 (Kisenge *et al*., 2007). Other clinical presentations that predicted CM were confusion (73%), social withdrawal (43%), seizures (35%), tachycardia (60%), meningismus (43%) and oral thrush (48%) (Kisenge *et al*., 2007).

The introduction of highly active anti-retroviral therapy (HAART) has decreased the progression of HIV to AIDS. ART administration decreases viral load by reducing viral replication. The ability of ART to thwart viral replication increases the number of CD4 cells in an HIV infected patient thereby revamping the immunity of the patient. This occurs as a result of reduction of plasma viral load to an undetectable level (Kelley *et al*., 2009). This also reduces development of opportunistic infections thereby cutting down on morbidity and mortality in HIV patients (Haynes *et al*., 1996; Antinori *et al*., 2009; Lartey *et al*., 2015).

The outcome of CM before and after the availability of HAART was studied in Uganda by Kambugu and colleagues (2008). They observed that 42% of the patients not on HAART (92) died in the first two weeks and by the sixth month, almost all the CM patients died (Kambugu *et al*., 2008). In the HAART era, 24 HIV patients were put on HAART with an initial median CD4 cell count of 20 cells/mm^3 but by six months of HAART, the median CD4 count increased to 66 cells/mm^3 and after 12 months of HAART, 18 of the 24 were alive. At six months of HAART, 15 of the 18 HIV patients had undetectable plasma HIV RNA (Ribonucleic acid) (Viral load). Nevertheless, the use of HAART at a late stage of CM may not be useful in reducing mortality according to Kambugu *et al*., (2008). They concluded that the use of ART may go a long way to increase CD4 cell count and lower viral load in an HIV

patient and can be good predictive values for the development and reduction of CM and CM related mortalities, respectively (Kambugu *et al*., 2008).

Early ART has a positive effect on CD4 count in HIV patients. In a cohort study of 366 HIV patients, 95% of patients who were on ART with a CD4 count equal or greater than 300 cells/mm^3, had an increase in CD4 count to 500 cells/mm^3 or more (Kelley *et al*., 2009). On the other hand, 44% and 25% of patients on ART with CD4 counts below 100 cells/mm^3 and from 100 to 200 cells/mm^3 did not have an increase of CD4 cell count to 500 cells/mm^3 or more over a long period of time (Kelley *et al*., 2009). This indicates that early usage of ART at a high CD4 cell count reduces the possibility of low immunity which eventually reduces opportunistic infections in HIV patients.

Haematological abnormalities are mostly associated with HIV infection leading to a high morbidity and mortality (Obirikorang and Yeboah, 2009; De Santis *et al*., 2011). In a cross sectional study, anaemia was very common in HIV positive women (20.5%) than HIV negative women (6.3%) (Munyazesa *et al*., 2012). Anaemia was higher in HIV patients with lower CD4 count with 7.6% and 32.2% of the patients having CD4 count equal or greater than 350 cells/mm^3 and below 200 cells/mm^3, respectively. Less than 4% of HIV positive patients had a marked haemoglobin (Hb) level below 10.0 g/dl with none in HIV negative patients. The mean WBC count in these HIV positive women was lower (3.7×10^3 cells/L) than in HIV-negative women (4.5×10^3 cells/L) with a mean WBC count lower in patients with CD4 count below 200 cells/mm^3. Thrombocytopenia was also common in the HIV positive women (13.5%) than the HIV-negative women (8.6%) although it could not be linked to lower CD4 count (Munyazesa *et al*., 2012).

HIV infection affects haematological parameters of patients irrespective of age, sex and HAART (Tagoe and Asantewaa, 2011). A mean Erythrocyte Sedimentation Rate (ESR) of

55.79 mm/hr was recorded in HIV positive patients as compared to HIV negative patients (3.68 mm/hr) (Tagoe and Asantewaa, 2011). Low mean Hb (10.2 g/dl) was seen in HIV positive patients compared to HIV negative patients (14.74 g/dl). Lower Total WBC and high mean platelet count were also recorded in the HIV positive subjects. Therefore, haematological parameters may be predictive values in ascertaining the progress and severity of HIV infection (Smith and Samadian, 1994). On the contrary, the use of HAART may have negative effect on haematological parameters. As reported by Lartey *et al*., (2015), anaemia cases of HIV admitted patients were caused by certain HAART like Zidovudin.

The use of HAART in HIV patients has been linked to insulin resistance, glucose intolerance and diabetes (Florescu and Kotler, 2007). Blood glucose abnormality in HIV patients on HAART is also associated with cardiovascular diseases (Florescu and Kotler, 2007).

2.3 THE CRYPTOCOCCUS ORGANISM

Cryptococcus species belongs to the division Basidiomycota (Ellis and Pfeiffer, 1990; Nester *et al.*, 2009). There are over 30 species of *Cryptococcus* but only *C. neoformans* usually affect humans and other animals. Others are *C. laurentti*, *C. adelensis*, *C. flavescens*, *C. uniguttulatus*, *C. magnur*, *C. humicolus*, *C. luteolus*, *C. macerans*, *C. uzbekistanesis*, *C. curvatus*, and they also have been implicated in clinical infections but in rare cases (Rosario *et al*., 2005).

C. neoformans, the species that causes cryptococcosis is a dimorphic fungus but differ from other dimorphic fungi due to the fact that they exist in the yeast phase in infected tissues and laboratory cultures (Kozubowski and Heitman, 2012). It exists in a filamentous form which is the sexual form and because of that in 1975, the organism was renamed *Filobasidiella*

neoformans although the earlier genus name is still generally used (Nester *et al*., 2009). This organism usually reproduces by budding (Kozubowski and Heitman, 2012).

C. neoformans is a gram-positive, round to oval yeast-like fungus measuring about 3 to 10 µm in diameter with a thick mucopolysaccharide capsule which serves as the organism's main virulent factor (Bicanic and Harrison, 2004; Nester *et al.*, 2009). The mucopolysaccharide capsule is made of glucuronoxylomanan and glucuronoxylomanaogalactan (Ramos *et al*., 2012).

Figure 2.1: **India ink preparation showing *Cryptococcus* species in a CSF specimen**

2.4 VIRULENCE FACTORS

The major virulence factors of *C. neoformans* are the mucopolysaccharide capsule (Nester *et al*., 2009), phenol oxidase enzyme and the organism's ability to grow at 37˚C (Bicanic and Harrison, 2004). The capsular mucopolysaccharides are similar in structure irrespective of serotypes. They are long, unbranched polymers made up of an α-1,3-linked polymannose backbone with β-linked monomeric branches of xylose and glucoronic acid (Mitchell, 2004). The phenol oxidase enzyme helps in the production of melanin, which prevents hydroxyl radical formation in the organism. This protects the organism from oxidase stress and host immune defense mechanisms (Casadevall *et al*., 2000; Eisenman *et al*., 2007).

2.5 ENVIRONMENTAL NICHE AND LIFE CYCLE

Cryptococcus neoformans can be found mainly in soil containing pigeon droppings or guano in humid environments. It is also found in faecal or cloacal samples of a dazzling array of avian species. Other varieties also inhabit the Eucalyptus trees (Ellis and Pfeiffer, 1990). *C. neoformans* is mainly associated with pigeon droppings (guano) whiles *C. gattii* is associated with the Eucalyptus trees and a dazzling array of tree species. *C. gattii* has also been isolated from water and air (Ellis and Pfeiffer, 1990; MacDougall *et al*., 2007)

C. neoformans is a dimorphic fungus but exists mainly in the yeast-like phase (Kozubowski and Heitman, 2012). It usually proliferates as saprophytic yeast lacking a capsule (Abegg *et al*., 2006). These yeast forms can transition to a filamentous form which gives rise to basidiospores which are small enough to enter the lungs during inhalation (Abegg *et al*., 2006; Kronstad *et al*., 2012). The spores and yeast mainly in a dessicated form serve as the infectious forms for man and animals (Abegg *et al*., 2006; Kronstad *et al*., 2012).

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2.6 EPIDEMIOLOGY

Although *C. neoformans* was first isolated from peach juice in 1894 (Speed and Dunt, 1995), there has been no outbreaks attributable to environmental sources, and no reports of animal-to-human as well as human-to-human transmission though the organism is numerously distributed worldwide in soil with accumulated pigeon droppings and also in vegetation (Nester *et al*., 2009). Before mid-1980, cryptococcal diseases were not common till the start of the AIDS epidemic. After the AIDS epidemic, prevalence of cryptococcal diseases increased to about 5 to 10% (Bicanic and Harrison, 2004). About 7 to 15% of HIV/AIDS patients develop cryptococcal infection around the world (Perfect and Casadevall, 2002) and according to Park *et al*., (2009), *Cryptococcus* spp is the fourth leading cause of death in sub-Sahara Africa. *C. neoformans* is the leading cause of meningitis in HIV/AIDS patients, with approximately 1 million cases of cryptococcal meningitis recorded worldwide each year (Park *et al*., 2009). This can be attributed to the high prevalence of HIV/AIDS in the sub-region. The overall mortality rate of cryptococcal meningitis ranges from 25 to 30%. For patients who may recover from CM, 40% may develop neurological disorders such as loss of vision, cranial nerve palsies, decreased mental function, and hydrocephalous with a quarter of patients having relapse occurring in them (Jain *et al*., 2005).

For every case of cryptococcal meningitis, millions of people are infected with the organisms without any symptoms or deleterious effect hence a symptomatic infection is often the first indication of AIDS (Nester *et al*., 2009). The global burden of cryptococcal meningitis is estimated at 9.6 million cases, resulting in 6.2 million deaths within three months of infection (Bicanic *et al.*, 2007; Park *et al*., 2009; La Hoz and Pappas, 2013).

Cryptococcal meningitis is prevalent among AIDS patients in Africa and Southeast Asia as compared to the United States which has little prevalence. This condition occurs sporadically

in Europe (Lortholary *et al.*, 2006). In a prospective, observational study with 340 HIV patients with CM symptoms in Nairobi, Kenya, it was documented that 33% of the study subjects had CM with an in-hospital mortality of 36% (Mdodo *et al.*, 2010).

In Kumasi, Ghana, *C. neoformans* was not identified in 28 HIV/AIDS subjects (Frimpong and Lartey, 1998). This may be due to scanty number of HIV/AIDS subjects being recruited for the research. A retrospective study in Kumasi with 117 patients of all ages showing meningitis symptoms but unknown HIV status, cultured bacteria (71.8%) and *Cryptococcus* (11.7%) were seen (Owusu *et al.*, 2012). The prevalence of *Cryptococcus* spp might have been higher than the reported prevalence if all patients were HIV/AIDS patients. Moreover, an increase in CM prevalence in Kumasi suggests that CM is gradually becoming one of the leading forms of meningitis in Ghana. On the other hand, exposure of the recruited HIV subjects to *C. neoformans* might have contributed to the varying outcomes of those researches in Kumasi.

Although it has been established that cryptococcosis is a prevalent opportunistic infection in HIV/AIDS patients, data on prevalence may be affected by time and geographical location. This may be due to the varying epidemiology of HIV infection and the AIDS management pattern (Dzoyem *et al.*, 2012). Furthermore, although the prevalence rate of cryptococcal disease is higher in certain geographical areas of the world like sub-Saharan Africa, there has been a tremendous decline in its prevalence (Mirza *et al.*, 2003). This has been attributed to the development of more effective antiretroviral therapy and prophylactic regimens used in the prevention of fungal infection (Powderly, 1993; Govender *et al.*, 2013).

In developed countries, like the USA, treatment of oral candidiasis with fluconazole and the administration of HAART to suppress the virus in the 1990s led to a tremendous decrease in

the annual incidence of cryptococcal diseases. In 1993, cryptococcal disease reduced from 66 cases per 1000 AIDS patients to 7 cases per 1000 patients in 2000 (Mirza *et al.*, 2003).

It is reported that cryptococcal meningitis is prevalent in men than women and less common in AIDS children (Likasitwattanakul *et al.*, 1999). In a study, 57.7% of 111 CM positive subjects were men (Mdodo *et al.*, 2010). Another study also reported 66% dominance by males (Dzoyem *et al.*, 2012) and the confirmed CM cases were within the age range of 26 to 45 years although some study subjects were below 18 years (Dzoyem *et al.*, 2012).

2.6.1 MOLECULAR AND SEROLOGICAL CHARACTERIZATION OF CRYPTOCOCCUS SPECIES AND DISTRIBUTION

The *Cryptococcus* species complex includes two basidiomycetous encapsulated yeast species, *C. neoformans*, an opportunistic pathogen, and *C. gattii*, a primary pathogen. Both species are common fungal agents of infection of the central nervous system. Based on the mucopolysaccharide capsule as an antigenic determinant, there are five serotypes of *C. neoformans*, with varying geographical and epidemiological characteristics (Bennett *et al.*, 1977). Serotype A (*C. grubii*) is found worldwide, and serotype D (*C. neoformans*) occurs mainly in Europe and South America. A hybrid of serotype AD of *C. neoformans* also exists, and is mostly found in the environment as well as in patients. *C. gattii* was previously known as *C. neoformans var. gattii* (serotype B and C), and thought to be restricted to tropical and subtropical zones (Xu and Mitchell, 2003). A recent outbreak of cryptococcosis on the Vancouver Island, Canada, has however expanded the range of this yeast to temperate regions (Fyfe *et al.*, 2008; Galanis and MacDougall, 2010).

A genotypic analysis employing PCR-fingerprinting, Amplified Fragment Length Polymorphisms (AFLP), Restriction Fragment Length Polymorphism (RFLP) and Multi-

locus Sequence Typing (MLST) has outlined four major molecular types of the *Cryptococcus* species complex. Both *C. neoformans* and *C. gatti* have four major molecular types; they are VNI, VNII, VNIII, VNIV, and VGI, VGII, VGIII, VGIV, respectively (Choi *et al.*, 2010; Olivares *et al.*, 2009). There is scanty information on the genetic diversity of Cryptococcus species in the African sub-region, and the serotype distribution in Accra is not known.

2.7 CLINICAL SIGNS AND SYMPTOMS

The commonest clinical presentations associated with CM are headache and fever which make it difficult to diagnose for several weeks because these symptoms are shown in a dazzling array of diseases (Othman *et al.*, 2004). The condition presents itself as pyrexia of unknown origin. Less common symptoms are nausea, vomiting and neck stiffness (Othman *et al.*, 2004; Lee *et al.*, 2011). Other less frequent symptoms include visual disturbances, cranial nerve palsies, seizures, confusion, photophobia, abnormal behaviour and focal neurological signs (McCarthy *et al.*, 2006; Govender *et al.*, 2013). In a research on Cryptococcosis by Rozenbaum and Gonçalves, (1994), where 171 subjects were recruited from 24 health institutions in Brazil, it was realized that 75% immunocompetent subjects had neck stiffness whilst AIDS subjects recorded 33% of the same symptoms.

In a retrospective study in Thailand, although clinical signs and symptoms were registered in both non-HIV and HIV patients admitted on the basis of CM, headache with fever and neck stiffness were shown a lot more in HIV patients (Tunlayadechanont *et al.*, 1997). Confirmed CM in HIV patients show signs and symptoms like headache, cough, night sweats, blurred vision, neck stiffness and altered mental status. Although HIV CM negative patients may

show similar clinical signs and symptoms, they were mostly seen in CM confirmed cases (Mdodo *et al*., 2010). Hence the use of clinical signs and symptoms in diagnosing CM in HIV patients may be reliable.

2.8 DIAGNOSIS AND DIAGNOSTIC TESTS

In several study, irrespective of study subject's immune status, CM diagnosis is done if one shows signs and symptoms suggestive of meningitis (Kumar *et al*., 2008; Cohen *et al*., 2010; Mdodo *et al*., 2010). Cryptococcal meningitis is usually diagnosed by identifying or detecting the organism, *C. neoformans*, in the CSF of a patient. The three arms for diagnosis of CM are culture (CSF and other body fluids), microscopy (India ink and Gram stain), and serology (Cryptococcal Antigen (CrAg) detection) (Kozel & Bauman, 2012).

2.8.1 DIRECT OBSERVATION

C. neoformans can sometimes be identified in CSF by direct observation using a microscope because of its unique round to oval shape, surrounded by a thick mucopolysaccharide capsule that stains strongly with Mayer's mucicarmine. One common example of a direct observational procedure is the India ink preparation which has been used in relatively scanty studies to diagnose CM (Saha *et al*., 2008).

In an India ink preparation, the capsule is seen as a clear halo around the yeast cell and the cytoplasm contains refractile inclusion bodies under the microscope. In the absence of budding, it can be confused with a fat droplet, lymphocytes or other artifact. India ink microscopy lacks sensitivity and requires experienced laboratory personnel (Saag *et al*., 2000; Perfect *et al*., 2010; WHO Rapid advice, 2011). The sensitivity of India ink preparation to ascertain the presence of *Cryptoccocus neoformans* may vary from 40 to 79 % (Butler *et al*., 1964; Sarosi *et al*., 1969; Sabetta and Andriole, 1985; Dismukes *et al*., 1987). In 294 specimens used (CSF, urine and blood), it was realized that the sensitivity of India ink

preparation was 8.5% (Dzoyem *et al*., 2012). Out of this percentage, 8% were from CSF India ink preparation. This suggests that CSF is a better specimen in the identification of *C. neoformans* in India ink preparation. In 57 HIV uninfected patients, India ink preparation was 100% sensitive (Chau *et al*., 2010). Hence the use of India ink in diagnosing CM may be reliable (Chau *et al*., 2010; Dzoyem *et al*., 2012).

Other useful stains in the identification of *Cryptococcus* spp are Gram's stain, Gomori methenamine silver, Alcian blue, Colloidal iron, Periodic acid-Schiff, Masson-Fontana silver stain, new methylene blue and Wright's stain. Little research on direct observation of *C. neoformans* using Gram's stain technique has been done. Gram technique was used as one of the diagnostic tools in a case report of a CM patient in India. Gram stain showed the presence of *C. neoformans* as gram-positive in the CSF (Arora and Aggarwal, 2013).

Although Gram's technique was employed in direct observation of CSF in Ghana, no information was provided on its sensitivity to diagnose CM (Owusu *et al*., 2012). However, Gram's stain can be used to couple India ink preparation in the identification of *C. neoformans* since the organism shows a typical gram-positive appearance. In CSF evaluation of patients with Cryptococcal meningitis in 136 patients, 83% were culture positive, while 84% were microscopy positive (Kambugu *et al*., 2008). This may suggest that microscopy may be sensitive occasionally than culture. Hence the use of microscopy may enhance the outcome of culture and serology.

2.8.2 CSF CULTURE

CSF culture is the gold standard to establish cryptococcal meningitis (Saha *et al*., 2008). This is because a definitive diagnosis of CM is done only by isolating the organism from CSF. In

order to have high yield or positive culture, several CSF specimens are collected and cultured at different times. Sabouraud's dextrose agar (SDA) and Nigger seed agar media may be used in isolating *C. neoformans*. The growth media should be devoid of cycloheximide since the organism's growth is inhibited by this antimicrobial agent. *C. neoformans* culture is incubated at 30°C to 32°C for about 72 hours (Edman, 1995).

Cultures can, however, be incubated for about 3 to 4 weeks before considering it negative if no growth is seen on the media (Edman, 1995). This is due to the fact that the organism may take a longer time to grow (Edman, 1995). In a retrospective study in North India, 36 of 40 (90%) recruits had their CSF culture positive for *C. neoformans* (Kumar *et al*., 2008). In 57 HIV uninfected patients, all the patients had positive CSF culture, representing 100% sensitivity (Kumar *et al*., 2008). This suggests how reliable culture can be in the diagnosis of cryptococcal meningitis. The viability of *C. neoformans* can be ascertained only when it is isolated by culture. This suggests why culture is the mainstay in CM diagnosis.

2.8.3 SEROLOGY

Serology to detect the capsular antigen is reliable. This is because healthy people are often seropositive. Cryptococcal antigen (CrAg) is shed into body fluids such as blood, CSF and urine. The concentration of CrAg is directly proportional and dependent on the stage of disease in the various specimens. Moreover, the presence of CrAg can be detected at high concentrations at 100 days before the start of the disease in about 11% of the people who develop cryptococcal disease making it possible for the diagnosis of asymptomatic people (Antinori, 2013).

The Cryptococcal Antigen Lateral Flow Assay has been reported to have an overall 99.5% and 98.8% sensitivity and specificity, respectively as compared to culture (Saha *et al.*, 2008).

To have a quick and reliable detection of *C. neoformans* and *C. gattii* infections, the use of CrAgLFA as compared to other diagnostic methods may be a better option. In detecting serotype C (*C. gattii*), CrAgLFA is more sensitive than other CrAg detection techniques (Kozel and Bauman, 2012).

In evaluating 136 CSF of patients with CM, out of 136 patients, 83% were cryptococcal culture positive, 84% were microscopy positive and 100% were CSF CrAg test positive (Kambugu *et al.*, 2008). This shows how culture and microscopy are less sensitive as compared to CrAg detection. Although the sensitivity of culture (79.3% in CSF only) was greater than India ink preparation (8.0% in CSF only) in a hospital-based surveillance, the use of CrAgLFA could have increased the diagnostic chances of the research, considering its high sensitivity (Dzoyem *et al.*, 2012).

2.8.4 CSF CELL COUNT AND CSF BIOCHEMISTRY

Meningitis by various pathogens causes changes in the structure of the meninges. This results into characteristic changes in the CSF values in meningitis patients. Inflammation causes loss of integrity of cerebral capillaries which leads to the loss of integrity of the blood-brain barrier. Loss of the integrity of the blood-brain barrier may result in leakage of protein, glucose consumption and increased migration of any of the leukocytes into the CSF. This is dependent on the aetiologic agent implicated in the inflammation of the meninges (de Vries *et al.*, 1997; Xu *et al.*, 2014).

Normal CSF contains 0-5 leukocytes/mm^3, mainly lymphocytes, though in neonates cell count may be up to 30/mm^3 (Bonadio, 1992). Generally, CSF of CM patients have white cell counts greater than 20/mm^3 with lymphocytes forming greater percentage (Perfect *et al.*, 2010; Arora and Aggarwal 2013; Patil *et al.*, 2013). CSF white cell count in HIV-associated

CM is lower and may even be normal or absent with an elevated CSF protein (Bicanic and Harrison, 2004; Perfect *et al*., 2010). A study by Cohen *et al*., (2010) saw low leukocytes count (Tables 2.1 and 2.2).

CSF glucose levels are used to differentiate bacterial and fungal meningitis from aseptic meningitis. Glucose levels are mostly decreased in bacterial and fungal meningitis whereas glucose levels are usually unaltered in aseptic meningitis (White and Fyles, 1950; Vengerov, 1979). Low CSF glucose level is due to the changes in the physiological functioning of the choroid epithelium as well as from the utilization by bacterial and/or fungal pathogens and leukocytes (Greenlee *et al*., 2004) (Tables 2.1 and 2.2).

The blood-brain barrier plays a major role in excluding proteins from the CSF. Proteins that have access to the CSF primarily enter the CSF by transport within pinocytotic vesicles traversing capillary endothelial cells (Machado *et al*., 2013). High protein level may be as a result of disruption of the blood-brain or blood-CSF barrier (Sellebjerg and Tumani, 2009). CSF protein may be high in CM patients, bacterial meningitis patients and tuberculous meningitis patients (White and Fyles, 1950; Sellebjerg and Tumani, 2009) (Tables 2.1 and 2.2).

Table 2.1: **Cerebrospinal fluid analysis for meningitis**

Etiology	Opening pressure	WBC Count	Protein (g/L)	Glucose (mmol/L)	CSF/Serum glucose ratio
Normal	<180 mmH_2O	≤5 cells/mm^3 Lymphocytes	0.15-0.45	2.5-4.4	0.6-0.7
Bacterial	>180 mmH_2O	>100 cells/mm^3 PMNs predominant	>0.45	<2.2	<0.4
Viral, ***T. pallidum***	<180 mmH_2O	25-500 cells/mm^3 Lymphocyte predominant	0.15-0.45	2.5-4.4	0.6-0.7
Fungal, ***M. tuberculosis,*** **Lymphoma**	Normal or increased	25-500 cells/mm^3 Lymphocytes predominant	>0.45	<2.2	<0.6

(Courtesy Roos and Brosch, 2012)

Table 2.2: **Reference values for CSF Biochemistry**

PARAMETER	REFERENCE
Glucose	2.5-4.5 mmol/L
Protein	0.15-0.45 g/L
Globulin	Negative

(Courtesy Central Lab Biochemistry Department SOP)

2.9 CM THERAPY

Because CM is a medical emergency, treatment is very crucial in abating its fatality. The first line drug for CM and other cryptococcosis is Amphotericin B. This antifungal agent has been used in treating the condition since the 1950s (Diamond *et al*., 1998). The use of antifungals and HAART increases the success rate of treating CM (Mirza *et al*., 2003). HIV replication can be controlled by administration of Highly Active Antiretroviral Therapy (HAART) which may increase the CD4 cell count (Kelley *et al*., 2009). The administration of antifungals should precede HAART administration to avoid Immune reconstitution inflammatory syndrome (Bicanic *et al*., 2009)

In developed countries, CM patients are managed with a combination of amphotericin B and flucytosine for the initial 2 weeks. This combination therapy has proven to have very high efficacy in treating CM condition (Nester *et al*., 2009).

In Ghana, suspected CM patients are often treated with Intravenous (IV) fluconazole. Treatment is done only when IV fluconazole is available and if the patient can afford it (Personal communication with Lartey). Oral fluconazole or itraconazole is used as a less effective alternative antifungal agent (Saag *et al*., 2000). Per the WHO guidelines, cryptococcal meningitis management comprises of an initial 2-week induction phase, followed by an 8-week consolidation phase, and then a long-term maintenance phase.

2.9.1 INDUCTION THERAPY

The optimal induction therapy for CM is two weeks of a combination therapy of intravenous Amphotericin B and flucytosine. Amphotericin B is given at a dose of 0.7 to 1.0 mg/kg/day while flucytosine is given at 100 mg/kg/day divided in four doses (25 mg/kg four times daily) (Perfect *et al*., 2010; WHO Rapid advice, 2011; Govender *et al*., 2013). In situations where

flucytosine is not available, oral fluconazole given at 800 mg/day can be used as a substitute in combination with amphotericin B (Bicanic *et al*., 2007; WHO Rapid advice, 2011). In a situation where patient is unable to afford amphotericin B or due to lack of facilities for blood monitoring, amphotericin B cannot be administered, high-dose fluconazole, given at 1200 mg/day plus flucytosine given at a dose 100 mg/kg/day is used (Saag *et al*., 1992; Van der Horst *et al*., 1997; WHO Rapid advice, 2011; Govender *et al*., 2013). In the absence of both amphotericin B and flucytosine, high-dose oral fluconazole, given at 1200 mg/day is recommended (Saag *et al*., 1992; Van der Horst *et al*., 1997; WHO Rapid advice, 2011; Govender *et al*., 2013). In the absence of flucytosine, amphotericin B can be administered alone at the same dose it is given in combination with flucytosine (Saag *et al*., 1992; WHO Rapid advice, 2011; Govender *et al*., 2013). Flucytosine is not administered as a monotherapy in the absence of amphotericin B and/or fluconazole because of the rapid resistance of the organism (Saag *et al*., 2000).

Amphotericin B is an intravenous fungicidal agent that is very efficacious in killing the causative organism of CM, *Cryptococcus* spp. Although it is efficacious, it is a very toxic antifungal agent which when administered would require its blood level to be monitored in the patient. This makes it difficult for its administration in centers where blood monitoring facilities are not available (WHO Rapid advice, 2011; Govender *et al*., 2013). Fluconazole, an oral antifungal agent, is the only option because it is less toxic and cheaper as compared to Amphotericin B. One major disadvantage of fluconazole is that it is fungistatic and would require the patients' immune system to mop the inhibited organism in the CSF (Bicanic *et al*., 2009). Amphotericin B-based therapy has a high treatment outcome as compared to fluconazole (Schaars *et al*., 2006; Lessells *et al*., 2011).

2.9.2 CONSOLIDATION AND MAINTENANCE PHASES

After the induction phase, an 8-week course of oral fluconazole, given at a dose of 400 mg/day for adults for the optimal consolidation phase of treatment (Van der Horst *et al*., 1997) followed up with a low-dose fluconazole, given at 200 mg/day for adults for the long-term maintenance phase (Saag *et al*., 1999; Govender *et al*., 2013).

CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY SITE AND DESIGN

This study was done in Accra at the Korle-Bu Teaching hospital (KBTH), the largest tertiary medical facility in Ghana (1600 bed capacity), and houses several specialized departments. Accra is the capital city of Ghana, and also the capital of the Greater Accra Region of Ghana. The Fevers' Unit (FU), an HIV care facility within KBTH, offers both outpatient and in-patient care to several dozens of patients on daily bases. The in-patient facility has a 24-bed capacity. This study was cross sectional.

3.2 SAMPLE SIZE

At 90% confidence interval with a population proportion of 33% (Mdodo *et al*., 2010) and allowable error of 10%, sample size was determined by the formula below;

$n=Z^2(P)(1-P)/e^2$, where Z, P and e are z-score, 90% confidence interval and allowable error respectively. n-minimum sample size.

That is $n=1.65^2\times (0.33)(1-0.33)/0.1^2$

n=60

3.3 SUBJECTS

Sixty-four HIV infected adults (≥18 years) with clinical presentations suggestive of meningitis or low CD4 count (≤100 cells/mm^3), reporting at the Fevers' Unit between August, 2014 to July, 2015 were enrolled. Out of these, 53 consented and lumbar punctures (LP) were performed. Persons below 18 years old were not included because Cryptococcal

meningitis is not prevalent in that age category. Patients already receiving antifungal therapy also were excluded.

3.4 SUBJECT INFORMATION

A structured questionnaire (Appendix III) was used to extract clinical data from patient folders/chart records. The information included patient demographics, previous history of hospitalization, other clinical conditions, ART therapy and when initiated, ART adherence status, last two serial CD4 counts, viral load, date of first diagnosis of HIV and type of HIV.

Subjects were examined by medical officers and clinical history including fever, meningismus, hypertensive, tachycardia, confusion, social withdrawal and wasting, were recorded.

3.5 CSF COLLECTION AND PROCESSING

Two CSF samples, 2 ml in a sterilized Bijoux bottle and 1 ml in a fluoride bottle were taken by trained Medical Doctors from each of the subjects. CSF samples in fluoride bottles were sent to the Biochemistry Department of the Central Laboratory for CSF glucose, total protein and globulin assays. CSF in Bijoux bottles were microbiologically analysed at the Medical Microbiology Department, SBAHS (MD-SBAHS).

A standard operating procedure (SOP) was developed for specimen processing, which was displayed boldly in the Research laboratory, of MD-SBAHS (Appendix I).

3.5.1 Macroscopic appearance of CSF

The colour, turbidity, presence of a coagulum or deposit of collected CSF were noted and documented. Normal CSF appearance was documented as clear and colourless, abnormal CSF appearances were documented as hazy, turbid, xanthochromic, blood-stained, presence of a coagulum or deposit.

3.5.2 Culture of CSF

For every specimen received, about 1.5 ml of CSF was aseptically transferred into a 2 ml eppendolf tube. The CSF specimen was centrifuged at 5000 rpm for 5 minutes in the eppendolf centrifuge tube. The supernatant was aseptically transferred into a cryotube and stored at a temperature of -20°C.

CSF sediment was cultured on Sabouraud's Dextrose Agar (SDA). SDA plate was incubated for 48-72 hours at 35˚C to 37°C, and observed for 14 days. Part of the CSF sediment was also inoculated in Brain Heart Infusion and 1% peptone water and incubated at 35˚C to 37˚C for 48 hours. After 48 hours, they were centrifuged at 5000 rpm for 5 minutes. The supernatants were discarded and the sediments were cultured on SDA.

3.5.3 CSF white cell count

A cover slip was put on an Improved Neubauer Counting Chamber. Five microlitres (5µl) of a well-mixed CSF was dispensed into the chamber beneath the coverslip. White cells that fell in the 4 quadrants plus 2 quadrants in the middle of the grid were counted and documented as the CSF white cell count.

3.5.4 CSF biochemistry

The colour, turbidity, presence of a coagulum or deposit of collected CSF were noted and documented. Normal CSF appearance was documented as clear and colourless, abnormal CSF appearance was documented as hazy, turbid, xanthochromic, blood-stained, presence of a coagulum or deposit. Blood stained specimens were centrifuged at 5000 rpm for 5 minutes and reported as clear supernatant plus red cell deposit, persistent colouration with or without red blood cells. Xanthochromic, hazy, turbid or cloudy, coagulum, fibrin clot were centrifuged at 5000 rpm for 5 minutes and the supernatant was used.

3.5.4.1 Total protein

Eight hundred microliters (800 µl) of 3% Trichloroacetic acid (TCA) was pipetted into three test tubes, namely the Reagent Blank, Standard and Test. Two hundred microliters (200 µl) of standard specimen and CSF specimen were pipetted into the Standard tube and Test, respectively. The content of the tubes were well mixed and incubated at room temperature for 10 minutes. A spectrophotometer was zeroed with reagent blank and the absorbance of the standard was read against the reagent blank and the test at 670 nm.

3.5.4.2 Glucose

Hundred microliters (100 µl) Glucose oxidase was pipetted into three test tubes, namely Reagent Blank, Standard and Test. One microliter (1 µl) of distilled water, 1 µl of peroxidase and also 1 µl of CSF specimen were pipetted into Reagent blank, Standard and Test, respectively. The contents of the three test tubes were mixed well and incubated for 15 minutes at room temperature for 7 minutes at 37˚C. The absorbance of standard and sample were measured against reagent blank at 505 nm. Glucose concentration of CSF specimen was calculated using the formula below;

Glucose= (Absorbance of Sample/Absorbance of Standard) ×Conc. of Standard (mmol/L)

3.5.4.3 Globulins

Four hundred to five hundred microliters (400 to 500 µl) of paandy's reagent was dispensed into a clean dry glass test tube. Two or 3 drops of CSF specimen were added to the tube and mixed well. The set up was observed for turbidity immediately against a contrasting background. A positive CSF globulin was read as turbidity observed immediately in the tube, whilst a negative CSF globulin was read as the absence of turbidity.

3.5.5 CSF Microscopy

Smears of well mixed CSF sediments were made on grease-free glass slides. They were air-dried, heat-fixed and subjected to Gram stain technique. They were then viewed under the microscope for gram positive round organisms occasionally with buds. Stained white cells were identified and documented as Polymorphonuclear neutrophils (PMNs) or lymphocytes.

Drops each of well mixed CSF sediments was placed on grease-free glass slides and a drop of India ink was added to each sediment. They were well mixed and thin films of the CSF sediment-India ink mixtures were prepared on the glass slides. Slides were air-dried and were viewed under the microscope using the oil immersion objective lens.

3.5.6 Cryptococcal Antigen Lateral Flow Assay (CrAgLFA)

A qualitative procedure was performed by adding one drop of Lateral Flow (LF) specimen Diluent (REF GLF025) to a cryotube. Forty microliters (40 µl) of uncentrifuged CSF specimen was added to the cryotube and mixed gently. The white end of the Cryptococcal Antigen Lateral Flow Test Strip (REF LFCR50) was put into the specimen and the setup was

left on the bench for 10 minutes. A positive result was read as both control and test lines turning red and a negative result was read and seen as the control line turning red (Fig 3.1). (www.immy.com)

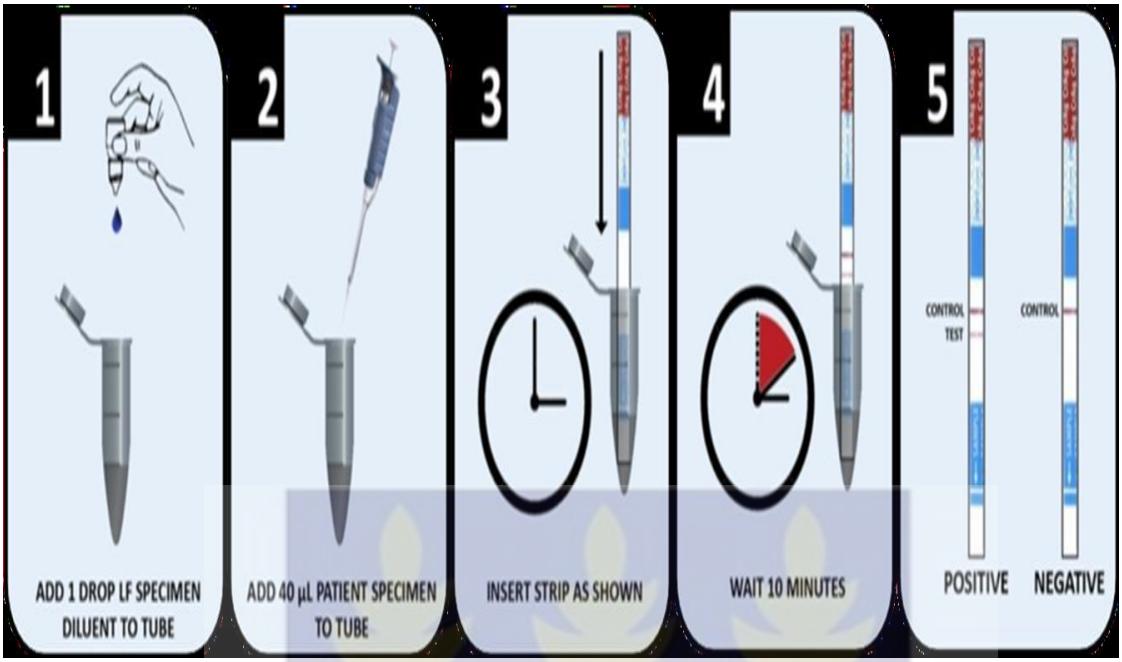

Figure 3.1: **CrAgLFA qualitative procedure**

(Courtesy www.immy.com)

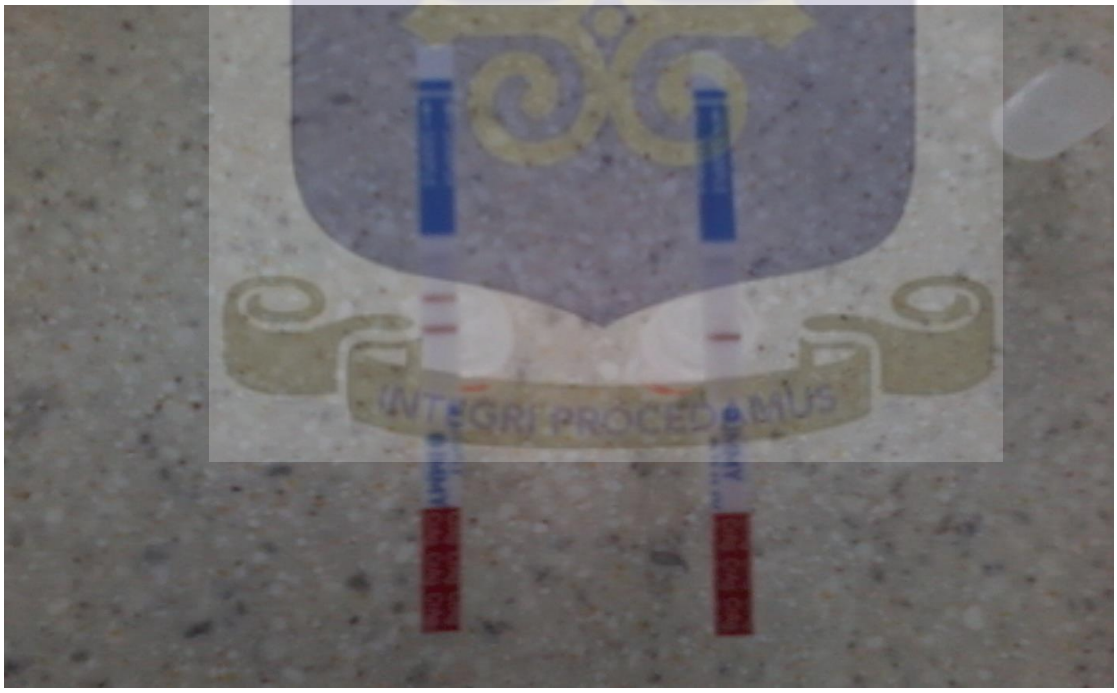

Fig 3.2: **CrAgLFA positive and negative set up in CSF specimens**

3.6 VIRAL LOAD SPECIMENS AND PROCESSING

About 4 ml of blood was phlebotomized from the patients and transferred into an EDTA vacutainer. It was centrifuged at 1000 rpm for 1 minute. The plasma was separated from the corpuscles and transferred into a cryotubes and stored at -80˚C. Plasma was thawed before use.

The viral loads of the participants were estimated using the COBAS®AmpliPrep/COBAS®TaqMan®HIV-1Test, Version 2.0. Eight hundred and fifty microliters (850 µl) plasmas were processed in the COBAS®AmpliPrep instrument by a generic silica-based capture technique to get a processed specimen that contained the HIV-1 RNA.

The processed specimens were then added to amplification mixtures in amplification tubes and heated to anneal downstream primers to the HIV-1 target RNA as well as to HIV-QS RNA to form a DNA strands complementary to the RNA target.

The reaction mixtures were heated using Thermal Cycler in the automated equipment to reverse transcribed and amplified target DNAs and cooled to denature RNA:cDNA hybrid. Double stranded DNAs were produced by the help of Thermus species Z05 DNA polymerase in the presence of Mn^{2+} and excess deoxynucleotides triphosphates. Automatic repeats of the process doubled the amount of double-stranded DNA. DNA strands containing deoxyuridines were destroyed by AmpErase enzyme leaving DNA containing deoxythymidine.

HIV-RNAs were detected using real-time PCR technology where dual-labelled fluorescent probes allowed for real-time detection of accumulated PCR products by monitoring the

emission intensity of fluorescent reporter dyes that were released during the amplification process (Damond *et al*., 2007).

3.7 DATA HANDLING AND ANALYSIS

Clinical notes and charts of suspected meningitis patients were audited and analysed. Both clinical and laboratory data were collected and recorded manually, and were entered into an Excel file template. Analysis of the data collected was done using SPSS VER. 20. Continuous data was presented as mean and standard deviation as well as range. Continuous variables were also presented using percentages.

3.8 ETHICS

Ethical approval for the study was sought from the Ethical and Protocol Review Committee of the UGMS (now SBHAS) (Appendix VII). The study was explained in the language of the patients who were well oriented and were aware of their environment, and were expected to give a written informed consent was obtained. For patients with impaired mental state, written informed consent was sought from the next of kin or immediate care giver. Patients were assigned study identification numbers (IDs) which were used throughout the study. The IDs were generated by using the initials of the disease under investigation and a chronological number according to how the patients were recruited. Laboratory tests were anonymized as these were required for routine patient care. Subjects were offered standard of care at the Fevers' Unit. All study documents were kept under lock and key and soft documents were secured by a password and only the PIs and Research assistants had access to study data. Patients benefitted from a calculated effort at identifying causative organisms in the CSF. This resulted in improved patient management. The CSF specimens were examined at no cost to patients.

CHAPTER FOUR

RESULTS

4.1 DEMOGRAPHIC CHARACTERISTICS OF STUDY SUBJECTS

This cross sectional study was done from August 2014 to July 2015. Sixty-four (64) suspected CM patients were enrolled but fifty-three (53) consented to be part of the study. Out of the fifty-three participants, 28 (53.8%) were males and 25 (47.2%) were females with an overall mean age of 40.7 years old. Twenty-nine (54.7%) of the participants were admitted within the last six months of the study. Majority of the subjects had secondary level education (Table 4.1).

4.2 CLINICAL PRESENTATION ON ADMISSION

Out of the 53 suspected CM cases, 39 (73.6%) complained of headache, and 26 (49.1%) had fever. Confusion/neurosymptoms was the second form of presentation by these patients. Nausea/vomiting 7 (13.2%), seizures 2 (3.5%), and stiff neck 7 (13.2%) were observed. Other clinical presentations like right facial palsy, slurred speech, diarrhoea, general malaise, neck pain, sudden unresponsiveness, dizziness, difficulty in swallowing, weight loss, cough, right limb weakness and right hemiparesis were seen in 35 (73.6%) of the subjects. Meningismus and tachycardia were seen in 12 (22.6%) and 23 (43.4%) of the suspected patients, respectively. Oral thrush was the major opportunistic infection seen in 22 (41.5%) of the subjects. The only CM confirmed subject showed headache, stiff neck and unstable gait as the only clinical presentations (Table 4.2).

TABLE 4.1: DEMOGRAPHIC CHARACTERISTICS OF STUDY SUBJECTS

CHARACTERISTIC	STUDY SUBJECTS N=53 (%)	CM POSITIVE n=1 (%)	CM NEGATIVE n=52 (%)
SEX			
Male	28 (52.8)	1 (100)	27 (51.9)
Female	25 (47.2)	0 (0)	25 (48.1)
AGE (in years)			
Mean±SD (Range)	40.7±7.7 (24-60)	31	40.9±7.7 (24-60)
EDUCATION			
Primary	12 (22.6)	1 (100)	11 (21.2)
Secondary	33 (62.3)	0 (0)	33 (63.5)
Tertiary	6 (11.3)	0 (0)	6 (11.5)
None	2 (3.8)	0 (0)	2 (3.8)
HOSP. ADMIS (within 6mnths)			
Yes	29 (54.7)	0 (0)	29 (55.8)
No	24 (45.3)	1 (100)	23 (44.2)
DATE OF HIV DIAGNOSIS		33 mnths	
<6mnths	16 (30.2)	0 (0)	16 (30.8)
6mnths-12mnths	24 (45.3)	0 (0)	24 (46.2)
>12mnths	7 (13.2)	1 (100)	6 (11.5)
Unknown	6 (11.3)	0 (0)	6 (11.5)

Mnths-Months
HOSP. ADMIS: Hospital admission

TABLE 4.2: CLINICAL PRESENTATIONS ON ADMISSION

CHARACTERISTICS	STUDY SUBJECTS N=53 (%)	CM POSITIVE n=1 (%)	CM NEGATIVE n=52 (%)
HEADACHE	39 (73.6)	1 (100)	38 (73.1)
FEVER	26 (49.1)	0 (0)	25 (48.1)
NAUSEA-VOMITING	7 (13.2)	0 (0)	7 (13.5)
SEIZURES	2 (3.5)	0 (0)	2 (3.8)
STIFF NECK	7 (13.2)	1 (100)	6 (11.5)
CONFUSION-NEUROSYMPTOMS	13 (24.5)	0 (0)	13 (25.0)
ORAL THRUSH	22 (41.5)	0 (0)	22 (42.3)
MENINGISMUS	12 (22.5)	0 (0)	12 (23.1)
TACHYCARDIA	23 (43.4)	0 (0)	23 (44.2)
OTHERS	35 (73.6)	1 (100)	35 (73.1)

Others: Right facial palsy, Slurred speech, Diarrhoea, General malaise, Neck pain, Sudden unresponsiveness, Dizziness, Difficulty in swallowing, Weight loss, Cough, Right limb weakness and right hemiparesis

4.3 CELL COUNTS IN STUDY SUBJECTS

Twenty (37.7%) of the patients had CD4 count below 100 cells/µl with the only CM confirmed case having a CD4 count of 182 cells/µl. The mean CD4 count of the study subjects was 180.5±228.5 (5-979 cells/µl). The mean viral load of the subjects was 351732.09±678638.84.

Forty-six (86.8%) of the subjects were in Stage IV of AIDS with about 96.3% having mild Glasgow coma score (Table 4.5). The WHO defined AIDS stage, viral load and Glasgow coma score of the CM confirmed case were Stage IV, 4.86×10^2 RNA copies/ml and mild (15/15), respectively.

54.7% of CSF WBC counts were below 5 cells/mm^3 and 22.6% were above 20 cells/mm^3. The mean CSF WBC counts was 19.8±48.8 (0-250). Lymphocytic pleocytosis was the highest (39.6%). CSF WBC differential of both polymorphonuclear neutrophils and lymphocytes was 9.4%. In relation to the CSF WBC differential of the CM confirmed case, 75% lymphocytes and 25% neutrophils were reported. CSF WBC count for this patient was 235 cells/mm^3 (Table 4.3).

TABLE 4.3: CELL COUNTS

PARAMETERS	STUDY SUBJECTS N=53 (%)	CM POSITIVE n=1 (%)	CM NEGATIVE n=52 (%)
WBC (cells/mm^3)			
Mean±SD	19.8±48.8	235	15.6±38.7
<5	29 (54.7)	0 (0)	29 (55.7)
5 to 20	12 (22.6)	0 (0)	12 (23.1)
>20	12 (22.6)	1 (100)	11 (21.2)
DIFFERENTIAL			
PMNs	7 (13.2)	0 (0)	7 (13.5)
Lymphocytes	21 (39.6)	0 (0)	21 (40.4)
PMNs/Lymph.	5 (9.4)	1 (100)	4 (7.7)
None	16 (30.2)	0 (0)	16 (30.8)
CD4 (most recent) µl			
Mean±SD	180.53±228.5	182	180.49±231.5
<100	20 (37.7)	0 (0)	20 (38.5)
>100	20 (37.7)	1 (100)	19 (36.5)
VIRAL LOAD (RNA copies/ml)			
Mean±SD	351732.1±678638.8	282	354330.5±686448.8
<100,000	21 (39.6)	0 (0)	21 (40.4)
>100,000	23 (43.3)	1 (100)	22 (42.3)
RNA-not detected	4 (7.5)	0 (0)	4 (7.7)
Not done	5 (9.4)	0 (0)	5 (9.6)

PMNs: Polymorphonuclear neutrophils
Lymph.: Lymphocytes

4.4 PERFORMANCE TEST FOR DIAGNOSTIC PARAMETERS USING CULTURE AS GOLD STANDARD

Of the fifty three CM suspected patients, only one was positive to CrAg test, India ink and Gram stain but not culture. This gives a prevalence of 1.9% among the subjects chosen. The sensitivity was zero for serology, India ink and Gram stain against culture. The specificity was 98.1% for CrAg, India ink and Gram stain against culture. The positive predictive values for CrAg, India ink and Gram stain was zero (Table 4.4).

TABLE 4.4: PERFORMANCE TEST FOR DIAGNOSTIC PARAMETERS USING CULTURE AS GOLD STANDARD

DIAGNOSTIC PARAMETERS	CULTURE	CrAgLFA	INDIA INK	GRAM STAIN	CI_{95}
ABSENT	53	52	52	52	
PRESENT	0	1	1	1	
PREVALENCE		1.9	1.9	1.9	
SENSITIVITY		0	0	0	0.0-6.9
SPECIFICITY		98.1	98.1	98.1	90.1-99.9
PPV		0	0	0	0.0-97.5
NPV		50.0	50.0	50.0	40.6-60.4

PPV=Positive predictive value
NPV=Negative predictive value
CI_{95}=95% confidence interval

4.5 OTHER CLINICAL INDICES OF STUDY SUBJECTS

Eleven (20.8%) of the participants were on antiretroviral therapy (ART). Out of this, 8 (15.1%) complied with ART treatment regimen. Forty-six (86.8%) of them were in WHO defined stage IV of AIDS with a mild Glasgow coma score (15/15) in 48 (90.6%).

Among the CM suspected subjects, 21 (39.6%) died after admission. The CM confirmed patient was alive the last time a follow up was made (Table 4.5).

TABLE 4.5: OTHER CLINICAL INDICES

INDICES	STUDY SUBJECTS N=53 (%)	CM POSITIVE n=1 (%)	CM NEGATIVE n=52 (%)
ON ART			
Yes	11 (20.8)	1 (100)	10 (19.2)
No	42 (79.2)	0 (0)	42 (80.8)
ART COMPLIANCE			
Yes	8 (15.1)	1 (100)	7 (13.5)
No	45 (84.9)	0 (0)	45 (86.5)
STAGING (current)			
II	1 (1.9)	0 (0)	1 (1.9)
III	4 (7.5)	0 (0)	4 (7.7)
IV	46 (86.8)	1 (100)	45 (86.5)
Unknown	2 (3.8)	0 (0)	2 (3.84)
GLASGOW COMA SCORE			
11 over 15	1 (1.9)	0 (0)	1 (1.9)
12 over 15	1 (1.9)	0 (0)	1 (1.9)
14/15	3 (5.7)	0 (0)	3 (5.8)
15/15	48 (90.6)	1 (100)	47 (90.4)
CLINICAL OUTCOME			
Dead	21 (39.6)	0 (0)	21 (40.4)
Discharged	32 (60.4)	1 (100)	31 (59.6)

4.6 CSF BIOCHEMISTRY PARAMETERS OF THE STUDY SUBJECTS

The mean value for CSF glucose for 50 specimens was 3.0±0.8 (1.1-4.2). Out of these specimens, 9 (17%) had CSF glucose values below 2.55 mmol/L. Four (7.5%) had CSF glucose above 4.0 mmol/L. A 2.8 mmol/L CSF glucose concentration was seen in the CM confirmed specimen with a CSF/Serum glucose ratio of 0.35 (lower than 0.6).

In respect to the CSF Total protein, a mean value of 1.1±1.7 (0.055-9.86) was recorded in 49 of the CSF specimen. Thirty two (60.4%) had CSF protein above 0.45 g/L. A CSF protein of 0.47 g/L was recorded in the CM confirmed specimen.

A negative globulin result was recorded in the CM confirmed CSF specimen. Thirty-one (58.5%) of the specimens were positive for globulin with 2 (3.8%) without any results due to insufficient specimen (Table 4.6).

TABLE 4.6: CSF BIOCHEMISTRY PARAMETERS

VARIABLE	STUDY SUBJECT N=53 (%)	CM POSITIVE n=1 (%)	CM NEGATIVE n=52 (%)
CSF GLUCOSE (mmol/L)			
Mean±SD	3.0±0.8	2.8	3.0±0.8
<2.5	9 (17.0)	0 (0)	9 (17.3)
≥2.5	41 (77.4)	0 (0)	40 (77.6)
CSF TOTAL PROTEIN (g/L)			
Mean±SD	1.09±1.65	0.47	1.10±1.66
≤0.45	17 (32.1)	0 (0)	17 (32.7)
>0.45	32 (60.4)	1 (100)	31 (59.6)
CSF GLOBULIN			
Positive	31 (58.5)	0 (0)	31 (59.6)
Negative	20 (37.7)	1 (100)	19 (36.5)

4.7 HAEMATOLOGICAL PARAMETERS OF THE STUDY SUBJECTS

Out of the 53 CM suspected subjects, 48 had their haemoglobin (Hb) level ascertained. The mean value was 9.6±2.2 (4.3-14.1). Forty-three (81.1%) had their Hb below 12.0 g/dl.

The Total WBC count had a mean value of 5.5±3.8 (1.13-24.07) in 48 subjects. Sixteen (30.2%) and 3 (5.7%) had WBC counts below 4.0×10^9/L and above 10.0×10^9/L respectively. A Total WBC count of 6.82×10^9 /L was seen in the CM confirmed case. The platelet count was high in 7 (13.2%) of the cases. On the other hand, 7 (13.2%) had low platelets counts.

Fifty- two of the participants had their Random blood glucose done with a mean of 7.9±3.2 (4.6-20.1 mmol/L) estimated. Although none of the RBS was low, 9 (17%) of the test results were high. An RBS of 8.0 mmol/L was recorded in the CM confirmed case.

In respect to the Erythrocyte Sedimentation Rate (ESR), 24 subjects had mean value of 83.7±37.1 (3.7-150), with 23 (43.4%) having high ESR (Table 4.7).

4.8 DRUGS ADMINISTERED TO STUDY SUBJECTS AFTER LUMBAR PUNCTURE

Thirty (56.6%) of the CM suspected subjects were administered with oral fluconazole. IV fluconazole was also administered to 5 (9.4%) of the participants. A total of 23 (43.4%) were put on antibiotics, 7 (13.2%) on anti-malarial and one on Anti koch's treatment. However, oral fluconazole was administered to the CM positive subject (Table 4.8).

TABLE 4.7: HAEMATOLOGICAL VARIABLES OF THE STUDY SUBJECTS

VARIABLES	STUDY SUBJECT N=53 (%)	CM POSITIVE n=1 (%)	CM NEGATIVE n=52 (%)
HAEMOGLOBIN (g/dL)			
Mean±SD	9.6±2.2	10.5	9.5±2.2
Range	(4.3-14.1)		(4.3-14.1)
<12	43 (81.1)	1 (100)	42 (80.8)
≥12	5 (9.4)	0 (0)	5 (9.6)
Not done	5 (9.4)	0 (0)	5 (9.6)
TOTAL WBC COUNT (/L)			
Mean±SD	5.5±3.8	6.8	5.5±3.8
Range	(1.1-24.1)		(1.1-24.1)
<4.0	16 (30.2)	0 (0)	16 (30.8)
4.0-10.0	29 (54.7)	1 (100)	28 (53.9)
>10.0	3 (5.7)	0 (0)	3 (5.8)
Not done	5 (9.4)	0 (0)	5 (9.6)
PLATELETS (/L)			
Mean±SD	229.8±140.8	129	232±141.6
Range	(36.0-714.0)		(36.0-714.0)
<100	7 (13.2)	0 (0)	7 (13.5)
100-300	28 (52.8)	1 (100)	27 (51.9)
>300	7 (13.2)	0 (0)	11 (21.2)
Not done	7 (13.2)	0 (0)	7 (13.5)
RBS (mmol/L)			
Mean±SD	7.9±3.2	8	7.9±3.2
Range	(4.6-20.1)		(4.6-20.1)
<3.6	0 (0)	0 (0)	0 (0)
3.6-11.0	43 (81.1)	1 (100)	42 (80.8)
>11.0	9 (17.0)	0 (0)	9 (17.3)
Not done	1 (1.9)	0 (0)	1 (1.9)
ESR (mm/hr)			
Mean±SD	83.6±37.1		83.7±37.1
Range	(3.7-150.0)		(3.7-150.0)
<30	1 (1.9)	0 (0)	1 (1.9)
>30	23 (43.4)	0 (0)	23 (44.23)
Not done	29 (54.7)	1 (100)	28 (53.85)

TABLE 4.8: DRUGS ADMINISTERED TO STUDY SUBJECTS AFTER LUMBAR PUNCTURE

DRUGS	**STUDY SUBJECT** N=53 (%)	**CM POSITIVE** n=1 (%)	**CM NEGATIVE** n=52 (%)
Oral fluconazole	30 (56.6)	1 (100)	29 (55.8)
IV fluconazole	5 (9.4)	0 (0)	5 (9.6)
Oral Azithromycin	4 (7.6)	0 (0)	4 (7.7)
Septrin	5 (9.4)	0 (0)	5 (9.6)
Sulphadoxine-Pyrimethamine	3 (5.7)	0 (0)	3 (5.8)
Pyrimethamine	3 (5.7)	0 (0)	3 (5.8)
Metronidazole	2 (3.8)	0 (0)	2 (3.9)
IV Metronidazole	1 (1.9)	0 (0)	1 (1.9)
Arthemeter Lumefantrine	1 (1.9)	0 (0)	1 (1.9)
Paracetamol	1 (1.9)	0 (0)	1 (1.9)
IV Ciprofloxacin	1 (1.9)	0 (0)	1 (1.9)
IV Metoclopramide	2 (3.8)	0 (0)	2 (3.9)
Anti Koch's	1 (1.9)	0 (0)	1 (1.9)
IV Ceftriaxone	6 (11.3)	0 (0)	6 (11.5)
Omeprazole	1 (1.9)	0 (0)	1 (1.9)
Oral Amoxiclav	1 (1.9)	0 (0)	1 (1.9)

CHAPTER FIVE

DISCUSSION

5.1 SUBJECTS BACKGROUND

This cross-sectional study to ascertain CM was done in adult-HIV subjects at the Fevers' Unit, KBTH. The age range of the subjects was between 24 to 60 years, with majority being males (28). This range is within the age range (18-80 years) of HIV patients reported by Lartey and colleagues (2015). Contrary to what Kumar and his team reported (2008) (100% prevalence), cryptococcal meningitis was confirmed in only 1.9% of these suspected patients.

5.2 CLINICAL PRESENTATIONS OF SUBJECTS ON ADMISSION

Clinical presentations in the current study were similar to those presented in HIV subjects with CM studies elsewhere (Kumar *et al*., 2008; Mdodo *et al*., 2010; Cohen *et al*., 2010); however, the clinical presentations in the current study were not predictive of CM. Headache, fever, nausea and vomiting, seizures and stiff neck were seen in most of the subjects with the CM confirmed patients showing headache, stiff neck and unstable gait. In the current study, oral thrush was a major opportunistic infection (41.5%) observed as was also reported in India (Kumar *et al*., 2008); however, oral thrush was not presented by the CM confirmed subject (Table 4.2).

Twenty-three of 53 of the enrolled subjects presented with tachycardia compared to 42 out of 109 and twenty-four (24) out of forty (40) in CM negative and CM positive patients, respectively reported by Kisenge and his colleagues (2007). Twelve (12) of fifty-three (53) presented with meningismus in the study subjects compared to twenty-eight (28) out of one hundred and nine (109) in CM negative patients and eighteen (18) out of forty-two (42) in CM positive patients

reported in a study (Kisenge *et al*., 2007). The only CM confirmed patient did not present with tachycardia and meningismus (Table 4.2).

5.3 CRYPTOCOCCAL MENINGITIS AND CD4 CELL COUNT

Although 37.7% of the CM subjects had CD4 count below 100 cells/µl, none was confirmed of having cryptococcal meningitis. The CM confirmed patient had a CD4 count of 182 cells/µl (Table 4.3). This portrays how CD4 count is not a good predictive value for CM in these suspected meningitis patients. This outcome is contrary to what was reported by Aslam and his colleagues in patients at the late stage of AIDS with CM (Aslam and Chandrasekhara, 2009). Although patients with very low CD4 did not predict CM, this may have accounted for the high mortality among these subjects (Table 4.5).

5.4 CSF WBC COUNT, CSF BIOCHEMISTRY AND CRYPTOCOCCAL MENINGITIS

In the present study, about 22% of the subjects had abnormal CSF WBC count exceeding 20 cells/mm^3 which is predictive of cryptococcal meningitis as reported by other studies (Schmidt *et al*., 1995; Perfect *et al*., 2010; Arora and Aggarwal 2013; Patil *et al*., 2013). Among the subjects of this study with pleocytosis, only one with CSF WBC count of 235 cells/mm^3 was confirmed of having CM. Greater proportion (39.6%) of these subjects had only lymphocytes in the CSF while 9.4% had both lymphocytes and neutrophils in their CSF. Lymphocytic predominance could predict CM as reported by other studies (Schmidt *et al*., 1995; Perfect *et al*., 2010; Arora and Aggarwal 2013; Patil *et al*., 2013). This type of abnormality confirmed CM in only one patient suggesting that CSF WBC count and differential may be poor diagnostic parameters for confirming CM.

Comparing the CSF leukocyte dominance of present study subjects to what was reported by Aslam and his colleague, lymphocytic predominance in this study formed majority (Aslam and Chandrasekhara, 2009). Lymphocytic pleocytosis confirmed CM in majority of their study subjects (Aslam and Chandrasekhara, 2009). CSF WBC count and CSF WBC differential could not predict CM in the current study except in the CM confirmed patient who had 235 cell/mm^3 with lymphocytic predominance. Since about 45% of the current study subjects had pleocytosis with only one confirmed of having Cryptococcal meningitis suggests that other aetiologic agents may be the cause of meningitis (Cohen *et al*., 2010).

Less than 20% of the subjects in the current study had low CSF glucose but a higher proportion (60.4%) of them had high CSF protein. The abnormalities in the CSF biochemistry and WBC count point at possible infection in the CSF, possibly CM. This deduction is in line with Sarosi and his colleagues' report that CM may present with low CSF glucose and high CSF proteins. On the contrary, the only CM confirmed subject in the current study had normal CSF glucose and slightly high CSF protein (Sarosi *et al*., 1969). Thirty-one patients had CSF globulin to be positive signifying possible infection. On the contrary, a negative CSF globulin negative was reported in the CM confirmed subject in the current subjects. This may suggest that CSF biochemistry may be misleading in diagnosing Cryptococcal meningitis.

5.5 HAEMATOLOGICAL PARAMETERS IN HIV PARTICIPANTS

In the current study, haemoglobin abnormality was seen in 81.1% of the subjects as compared to 39.6% and 47.5% haemoglobin abnormalities in HIV-negative/CM negative (Tagoe and Asantewaa, 2011) and HIV-positive/CM positive subjects (Kumar *et al*., 2008), respectively. The mean HB was low (9.6 g/dl) in the current study subjects which was not different from 10.20 g/dl in HIV positive patients as compared to a mean HB of 14.74 g/dl in HIV negative

patients as reported by Tagoe and Asantewaa (2011). This implies that haemoglobin level in HIV patients is lower than HIV negative patients.

Greater proportion (43.4%) of our study subjects had ESR values above 30 mm/hr similar to other reported cases in Ghana (Tagoe and Asantewaa, 2011). ESR levels are high in HIV positive patients compared to HIV negative patients.

There was high WBC in 3 (5.7%) of the HIV patients in the current study. In other studies, high WBC was not reported in HIV patients (Tagoe and Asantewaa, 2011). Sixteen (30.2%) of the subjects in the current study had low WBC as compared to 40 (26.8%) in a study by Tagoe and Asantewaa. The immunity status of the current subjects can be defined by their low WBC but it is a poor indicator as compared to CD4 because only 54.7% of the subjects had normal WBC level compared to the abnormality in CD4 in majority of the subjects. The CM confirmed subject had normal WBC with an abnormal CD4 (Tables 4.3 and 4.7).

Platelet count was low in seven (13.2%) and high in 7/53 (13.2%) of the current subjects as compared to 13/149 (8.7%) and 2/149 (1.3%), respectively reported by Tagoe and Asantewaa (2011). This signifies less platelet abnormality in HIV patients. Since smaller proportion of the current subjects was on ART, haematological abnormalities may be attributed to the effect of HIV itself (Lartey *et al*., 2015).

Nine (17.0%) of the current subjects had high blood glucose level. Hyperglycaemia can be induced by ART usage (Florescu and Kotler, 2007). The proportion of current subjects on ART (20.8%) did not differ greatly from those with high blood glucose level (17.0%). Hyperglycaemia in the subjects may be caused by ART. On the other hand, the CM confirmed patient on ART had normal blood glucose level. The only reason could be the patient had not been on ART for a longer period of time to have an ART induced hyperglycaemia (Florescu and Kotler, 2007)

5.6 DIAGNOSTIC PROCEDURES USED AND RATE OF POSITIVITY

In respect to the 53 HIV subjects recruited in this study, none was confirmed positive for CM using culture. One subject was confirmed positive for CM using serology (CrAgLFA), India ink, and Gram's stain. This gives a CM prevalence of 1.9% among our subjects. Conflicting CM prevalence have been reported in Ghana, 0% (Frimpong and Lartey, 1998) and 11.7% (Owusu *et al*., 2012). The current study recorded CM prevalence of 1.9% which is lower than the work done by Owusu and his team (2012).

In the sub-Saharan region, a prevalence of 33% was reported in Kenya (Mdodo *et al*., 2010). The sensitivity per calculation for serology, India ink and Gram stain were zero when compared to culture as gold standard although these parameters were able to detect the presence of the fungus. Contrary to the low sensitivity of India ink and Gram stain microscopy, the ability of these parameters to detect the presence of the fungus in the CSF suggest that there was a high burden of the organism in the CSF although the patient was on ART (Mdodo *et al*., 2010; Bicanic and Harrison, 2004).

5.7 ANTIRETROVIRAL THERAPY, VIRAL LOAD, CD4 CELL COUNT AND CM

Although recruited subjects showed clinical presentations suggestive of meningitis, possibly CM, most recent CD4 count (≤100 cells/µl), and high viral load of patients who are not on ART could not be used as predictive values for CM. This deduction is due to the low CM prevalence in the subjects. This outcome is not in line with other studies (Cohen *et al*., 2010; Mdodo *et al*., 2010). About 80% of the CM negative subjects were ART naïve with high viral load. This might have accounted for the general low CD4 cell count with 37.7% below 100 cells/mm^3 loads (Table 4.3). The low CD4 cell count defines the current staging of the participants where 45 (86.5%) were in the WHO defined stage IV.

The only CM confirmed subject was on ART. The current CD4 count and current staging were 182 cells/µl and stage IV, respectively. The CM positive subject had an initial CD4 count of 214 cells/µl. This shows that the CD4 kept reducing. This suggests that the CM positive subject had been on ART for a short period of time at a low CD4. When ART is started early at a high CD4 for a long time, there is a greater possibility of CD4 increase (Kelley *et al*., 2009). This is in line with what Kelley and his team reported (Tables 4.3 and 4.5). Although the CD4 count was above 100 cells/µl, the immunity of the CM positive subject (182 cells/µl) will still be considered as low. A very low viral load of 486 RNA copies/µl was seen in the CM positive subject (Table 4.3). The low viral load did not match the low CD4 count of the subject suggesting possible underlying condition accounting for the low CD4 count.

5.8 MORTALITY RATE IN RELATION TO CD4 CELL COUNT AND HAEMATOLOGICAL PARAMETERS

Although suspected CM patients in this study were put on oral fluconazole, IV fluconazole, antibiotics, antimalarial and analgesic as prophylaxis, mortality rate was high. This may be due to patients accessing the unit at a late stage.

The high mortality rate of about 40% recorded in the present study may be due to the general low CD4 cell count (37.7% below 100 cells/mm^3 and 18.8% between 100 - 200 cells/mm^3). The high mortality rate may also be attributed to low haemoglobin level recorded in 81.1% of the study subjects which could be as a result of the direct effect of the virus (Lartey *et al*., 2015; Obirikorang and Yeboah, 2009). Low haemoglobin level and Total WBC count among the current subjects may be used as indicators for low CD4 count (Munyazesa *et al*., 2012). Few of the death cases among the subjects on ART could be caused by ART induced hyperglycaemias which were not managed (Florescu and Kotler, 2007).

The mortality rate of these subjects (40.4%) was within the same range (20% to 42%) as reported in CM confirmed patients in Uganda by Kambugu and his team and higher than in CM negative patients (34.0%) as reported by Mdodo and his team (Kambugu *et al.*, 2008; Mdodo *et al.*, 2010). The CM confirmed subject in this study was put on oral fluconazole. The patient was discharged and still alive the last time a follow-up was made. This suggests that oral fluconazole may be efficacious against *Cryptococcus* spp.

5.9 FACTORS ACCOUNTING FOR LOW CRYPTOCOCCAL MENINGITIS PREVALENCE AMONG STUDY SUBJECTS

A very low prevalence was recorded although this study was to ascertain the prevalence of CM among hospitalized patients with HIV, a major risk factor for CM development. This may be attributed to whether these patients were exposed to and also the length of time of exposure to *C. neoformans* (Fessel, 1993). An HIV patient living in an area with high population of pigeons and other avians for a very long time may stand a high chance of developing CM as compared to an HIV patient living in an area with few or no pigeons or avians (Othman *et al.*, 2004).

The virulence nature of the organism may also play a role in this situation. The inability of inhaled *C. neoformans* basidiospores to produce capsule will make them vulnerable to phagocytosis. Although these subjects had defect in greater percentage of T-lymphocytes, there was the possibility that alveolar macrophages and CSF macrophages might have internalized and destroyed the organisms (Gilbert *et al.*, 2014; Bicanic and Harrison, 2004; Nester *et al.*, 2009).

One major exclusion criteria was to disqualify inpatients on antifungals, but because most of these patients were diagnosed at the later stage of HIV/AIDS, self-medication using antifungal to treat diseases like oral thrush prior to hospitalization was a possibility. This suspicion was made because 75.5% of these participants were diagnosed of having HIV within the last six to twelve

months (Table 4.1). There is also the suspicion that some of the subjects might have been attending clinic elsewhere and had to relocate from their communities to avoid stigmatization. Furthermore, 54.8% of these suspected CM patients were admitted within the last six months and this increases the possibility of these subjects being issued with oral fluconazole as prophylaxis over the period before accessing the Fevers' Unit, Korle-Bu. This might have accounted for the low prevalence of CM and inability of the organism to be cultured in the only serologically and microscopically CM positive HIV patient. This is not different from what Mirza and his team reported (Mirza *et al*., 2003). The ability of microscopy (India ink and Gram's stain) to detect the presence of the organism/s that was culture negative in the CSF of the CM confirmed patient may suggest that oral fluconazole might have had a fungistatic effect on the organism (Bicanic *et al*., 2009).

5.10 LIMITATIONS OF THE STUDY

Limited number of study subjects was enrolled during the one year study period. Several factors might predict CM if a larger study population was used.

I could not verify whether subjects had self-medicated with anti-fungal agents before recruitment.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

There was low prevalence of cryptococcal meningitis among the study subjects which suggests that CM may not necessarily be the leading form of meningitis in HIV/AIDS subjects at the Fevers' Unit, Korle-Bu, although AIDS is a major risk factor in developing CM. Other factors may play role in the development of CM in HIV/AIDS patients.

Although serology, India ink and Gram stain microscopy were able to detect one positive case, their individual sensitivities were low (0%). The low sensitivities could be as a result of the detection of a single positive case which was not detected by culture, the gold standard. On the other hand, their individual specificities were high (98.1%).

Since CSF WBC count, CSF biochemistry, high viral load, CD4 counts below 100 cells/mm^3 could not predict CM in the study subjects, they may predict the presence of other etiologic agents. The use of clinical presentations in diagnosing Cryptococcal meningitis could be misleading.

6.2 RECOMMENDATIONS

In respect to the low prevalence of Cryptococcal meningitis in our HIV/AIDS subjects, it would be prudent to ascertain the possible causative organism/s of meningitis in these subjects.

Molecular detection using polymerase chain reaction (PCR) of *Cryptococcus neoformans* must be done on CSF specimens. This is to compare the sensitivity and specificity of PCR to culture, serology, India ink and Gram stain.

Culture should be coupled with CrAg testing, India ink and gram stain microscopy to increase the chances of diagnoses of CM suspected cases.

In respect to the limited number of subjects, other immunocompromised patients such as those undergoing organ transplants, diabetes mellitus patients showing meningitis symptoms should be recruited. Furthermore, subjects should include other HIV/AIDS patients from other neighbouring hospitals.

A study should be undertaken to find out if *Cryptococcus* species exist in our environment. This can be done by taking cloacal swabs, pigeon guano, poultry birds' droppings as well as soil samples and decaying trees.

REFERENCES

Abegg, M. A., Cella, F. L., Faganello, J., Valente, P., Schrank, A., and Vainstein, M. H. (2006). *Cryptococcus neoformans* and *Cryptococcus gattii* isolated from the excreta of psittaciformes in a Southern Brazilian Zoological Garden. *Mycopathologia*, 161(2): 83-91.

Andama, A. O., den Boon, S., Meya, D., Cattamanchi, A., Worodria, W., Davis, J. L.,Walter, N.D. and Huang, L. (2013). Prevalence and outcomes of cryptococcal antigenemia in HIV-seropositive patients hospitalized for suspected tuberculosis in Uganda. *Journal of Acquired Immune Deficiency Syndromes*, 63(2): 189-94.

Anderson, N. E., and Willoughby, E. W. (1987). Chronic meningitis without predisposing illness—a review of 83 cases. *QJM*, 63(1): 283-295.

Antinori, S. (2013). New Insights into HIV/AIDS-Associated Cryptococcosis. *Isrn Aids*, 2013: 1-22.

Antinori, S., Ridolfo, A. L., Fasan, M., Magni, C., Galimberti, L., Milazzo, L., Sollima and Parravicini, C. (2009). AIDS-associated cryptococcosis: A comparison of epidemiology, clinical features and outcome in the pre- and post-HAART eras. Experience of a single centre in Italy. *HIV Medicine*, 10(1): 6–11.

Arora, U., and Aggarwal, A. (2013). Cryptococcal meningitis in AIDS patients - a report of two cases. *Indian Journal of Medical Microbiology*, 19(3): 153-154.

Asboe, D., Aitken, C., Boffito, M., Booth, C., Cane, P., Fakoya, A., Gerretti, A.M, and Yirrell, D. (2012). British HIV Association guidelines for the routine investigation and monitoring of adult HIV-1-infected individuals in 2011. *HIV Medicine*, 13(1): 1-44.

Aslam, S. M. S., and Chandrasekhara, P. (2009). Study of Cryptococcal meningitis in HIV seropositive patients in a tertiary care centre. *Journal, Indian Academy of Clinical Medicine*, 10(3): 111.

Berenguer, J., Moreno, S., Laguna, F., Vicente, T., Adrados, M., Ortega, A., Gonzalez-LaHoz, J and Bouza, E. (1992). Tuberculous meningitis in patients infected with the human immunodeficiency virus. *New England Journal of Medicine*, 326(10): 668-672.

Beyrer, C., and Abdool Karim, Q. (2013). The changing epidemiology of HIV in 2013. *Current Opinion in HIV and AIDS*, 8(4): 306-310.

Bicanic, T., Brouwer, A. E., Meintjes, G., Rebe, K., Limmathurotsakul, D., Chierakul, W.,Teparrakkul, P., and Harrison, T. (2009). Relationship of cerebrospinal fluid pressure, fungal burden and outcome in patients with cryptococcal meningitis undergoing serial lumbar punctures. *Aids*, 23(6): 701-706.

Bicanic, T., and Harrison, T. S. (2004). Cryptococcal meningitis. *British Medical Bulletin*, 72: 99-118.

Bicanic, T., Meintjes, G., Wood, R., Hayes, M., Rebe, K., Bekker, L.-G., and Harrison, T. (2007). Fungal burden, early fungicidal activity, and outcome in cryptococcal meningitis in antiretroviral-naive or antiretroviral-experienced patients treated with amphotericin B or fluconazole. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, 45(1): 76-80.

Bonadio, W. A. (1992). The cerebrospinal fluid: physiologic aspects and alterations associated with bacterial meningitis. *The Pediatric infectious disease journal*, 11(6): 423-432.

Boulware, D. R., Rolfes, M. A., Rajasingham, R., von Hohenberg, M., Qin, Z., Taseera, K., Schutz, C., and Meya, D. B. (2014). Multisite validation of cryptococcal antigen lateral flow assay and quantification by laser thermal contrast. *Emerging infectious diseases*, 20(1): 45.

Brouwer, M., Tunkel, A., R., and van de Beek, D. (2010). Epidemiology, Diagnosis, and Antimicrobial Treatment of Acute Bacterial Meningitis. *Clin Microbiol Rev*, 23: 467-492.

Butler, W. T., Alling, D. W., Spickard, A., and Utz, J. P. (1964). Diagnostic and prognostic value of clinical and laboratory findings in cryptococcal meningitis: a follow-up study of forty patients. *New England Journal of Medicine*, 270(2): 59-67.

Carbonnelle, E. (2009). Laboratory diagnosis of bacterial meningitis: usefulness of various tests for the determination of the etiological agent. *Med Mal Infect.*, 39: 581-605.

Casadevall, A., Rosas, A. L., and Nosanchuk, J. D. (2000). Melanin and virulence in Cryptococcus neoformans. *Current opinion in microbiology*, 3(4): 354-358.

Chadwick, D. R. (2005). Viral meningitis. *British medical bulletin*, 75(1): 1-14.

Chau, T. T., Mai, N. H., Phu, N. H., Nghia, H. D., Chuong, L. V, Sinh, D. X., Duong, V.A and Day, J. N. (2010). A prospective descriptive study of cryptococcal meningitis in HIV uninfected patients in Vietnam - high prevalence of Cryptococcus neoformans var grubii in the absence of underlying disease. *BMC Infectious Diseases*, 10: 199.

Choi, Y. H., Ngamskulrungroj, P., Varma, A., Sionov, E., Hwang, S. M., Carriconde, F., Meyer, W and Kwon-Chung, K. J. (2010). Prevalence of the VNIc genotype of *Cryptococcus neoformans* in non-HIV-associated cryptococcosis in the Republic of Korea. *FEMS Yeast Research*, 10(6): 769-778.

Cohen, D. B., Zijlstra, E. E., Mukaka, M., Reiss, M., Kamphambale, S., Scholing, M., Waitt, P.I. and Neuhann, F. (2010). Diagnosis of cryptococcal and tuberculous meningitis in a resource-limited African setting. *Tropical Medicine and International Health*, 15(8): 910-917.

Damond, F., Roquebert, B., Benard, A., Collin, G., Miceli, M., Yeni, P., Brun-Vezinet, F., and Descamps, D. (2007). Human immunodeficiency virus type 1 (HIV-1) plasma load discrepancies between the Roche COBAS AMPLICOR HIV-1 MONITOR Version 1.5 and the Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 assays. *Journal of clinical microbiology*, 45(10): 3436-3438.

Desalermos, A., Kourkoumpetis, T. K., and Mylonakis, E., (2012). Update on the epidemiology and management of cryptococcal meningitis. *Expert Opinion on Pharmacotherapy*, 13(6): 783-789.

De Santis, G. C., Brunetta, D. M., Vilar, F. C., Brandao, R. A., de Albernaz Muniz, R. Z., de Lima, G. M. N., Amorelli-Chacel, M.E., Covas, D.T., & Machado, A. A. (2011). Hematological abnormalities in HIV-infected patients. *International Journal of Infectious Diseases*, 15(12): e808-e811.

de Vries, H. E., Kuiper, J., de Boer, A. G., Van Berkel, T. J., & Breimer, D. D. (1997). The Blood-Brain Barrier in Neuroinflammatory. Pharmacological reviews, 49(2): 143-156.

Diamond, D. M., Bauer, M., Daniel, B. E., Leal, M. A. E., Johnson, D., Williams, B. K., Thomas, A.M. and Larsen, R. A. (1998). Amphotericin B colloidal dispersion combined with flucytosine with or without fluconazole for treatment of murine cryptococcal meningitis. *Antimicrobial Agents and Chemotherapy*, 42(3): 528-533.

Dismukes, W. E. (1988). Cryptococcal meningitis in patients with AIDS. *Journal of Infectious Diseases*, 157(4): 624-628.

Dismukes, W. E., Cloud, G., Gallis, H. A., Kerkering, T. M., Medoff, G., Craven, P. C., Kaplowitz, L.G., and Blackwelder, W. C. (1987). Treatment of cryptococcal meningitis with combination amphotericin B and flucytosine for four as compared with six weeks. *New England Journal of Medicine*, 317(6): 334-341.

Dzoyem, J. P., Kechia, F. A., Ngaba, G. P., Lunga, P. K., and Lohoue, P. J. (2012). Prevalence of cryptococcosis among HIV-infected patients in Yaounde, Cameroon. *African Health Sciences*, 12(2): 129-133.

Edman J. C. (1995). Medical mycology. In: Jawetz, Melnick and Alderberg's medical microbiology. 20th Ed. London: Prentice Hall International (UK): 649-650.

Eisenman, H. C., Mues, M., Weber, S. E., Frases, S., Chaskes, S., Gerfen, G., and Casadevall, A. (2007). *Cryptococcus neoformans* laccase catalyses melanin synthesis from both D- and L-DOPA. *Microbiology*, 153(12): 3954-3962.

Ellis, D. H., and Pfeiffer, T. J. (1990). Ecology, life cycle, and infectious propagule of *Cryptococcus neoformans*. *Lancet*, 336(8720): 923-925.

Ellis, D. H., and Pfeiffer, T. J. (1990). Natural habitat of *Cryptococcus neoformans var. gattii*. *Journal of Clinical Microbiology*, 28(7): 1642-1644.

Fang, C. T., Hsu, H. M., Twu, S. J., Chen, M. Y., Chang, Y. Y., Hwang, J. S., Wang, J. D and Chuang, C. Y. (2004). Decreased HIV transmission after a policy of providing free access to highly active antiretroviral therapy in Taiwan. *The Journal of Infectious Diseases*, 190(5): 879-885.

Feldman, W. E. (1977). Relation of concentrations of bacteria and bacterial antigen in cerebrospinal fluid to prognosis in patients with bacterial meningitis. *New England Journal of Medicine*, 296(8): 433-435.

Fessel, W. J. (1993). Cryptococcal meningitis after unusual exposures to birds. *New England Journal of Medicine*, 328(18): 1354-1355.

Florescu, D., and Kotler, D. P. (2006). Insulin resistance, glucose intolerance and diabetes mellitus in HIV-infected patients. *Antiviral therapy*, 12(2): 149-162.

French, N., Gray, K., Watera, C., Nakiyingi, J., Lugada, E., Moore, M., Lalloo, D., Whitworth,J.A.G. and Gilks, C. F. (2002). Cryptococcal infection in a cohort of HIV-1-infected Ugandan adults. *AIDS (London, England)*, 16(7): 1031-1038.

Frimpong, E., and Lartey, R. A. (1998). Study of the aetiologic agents of meningitis in Kumasi, Ghana, with special reference to *Cryptococcal neoformans*. *East Afr Med J*, 75: 516-519.

Fyfe, M., MacDougall, L., Romney, M., Starr, M., Pearce, M., Mak, S., Mithani, S. and Kibsey, P. (2008). *Cryptococcus gattii* infections on Vancouver Island, British Columbia, Canada: emergence of a tropical fungus in a temperate environment. *Canada Communicable Disease Report*, 34(6): 1-12.

Galanis, E., & MacDougall, L. (2010). Epidemiology of *Cryptococcus gattii*, British Columbia, Canada, 1999-2007. *Emerging Infectious Diseases*, 16(2): 251-257.

Garber, G. (2001). An overview of fungal infections. *Drugs*, 61(1): 1-12.

Garges, H. P., Moody, M. A., Cotten, C. M., Smith, P. B., Tiffany, K. F., Lenfestey, R., Li, J. S Fowler, V. G., and Benjamin, D. K. (2006). Neonatal meningitis: what is the correlation

among cerebrospinal fluid cultures, blood cultures, and cerebrospinal fluid parameters?. *Pediatrics*, 117(4): 1094-1100.

Gilbert, A. S., Wheeler, R. T., May, R. C., Whitney, L. C., Bicanic, T., Sil, A., and Taylor, J. W. (2014). Fungal Pathogens : Survival and Replication within Macrophages Fungal Pathogens : Survival and Replication within Macrophages. *Cold Harbor perspective in medicine*, 5: a019661.

Ginsberg, L., (2004). Difficult and recurrent meningitis. *Journal of Neurology, Neurosurgery and Psychiatry*, 75(suppl 1): i16-i21.

Glass, J. P., Melamed, M., Chernik, N. L., and Posner, J. B. (1979). Malignant cells in cerebrospinal fluid (CSF) the meaning of a positive CSF cytology. *Neurology*, 29(10): 1369-1375.

Goodman, J. S., Kaufman, L., and Koenig, M. G. (1971). Diagnosis of cryptococcal meningitis: value of immunologic detection of cryptococcal antigen. *New England Journal of Medicine*, 285(8): 434-436.

Govender, N., Meintjes, G. A, Bicanic, T., Dawood, H., Harrison, T., Jarvis, J., Karstaedt, A.S. and Scriven, J. (2013). Guideline for the prevention, diagnosis and management of cryptococcal meningitis among HIV-infected persons: *Sajhivmed*, 14(2): 76-86.

Greenlee, J. E., and Carroll, K. C. (2004). Cerebrospinal fluid in central nervous system infections. *Infections of the central nervous system*.

Gugnani, H. C., Mitchell, T. G., Litvintseva, A. P., Lengeler, K. B., Heitman, J., Kumar, A., Basu, S. and Paliwal-Joshi, A. (2005). Isolation of *Cryptococcus gattii* and *Cryptococcus neoformans var. grubii* from the flowers and bark of Eucalyptus trees in India. *Medical*

Mycology : Official Publication of the International Society for Human and Animal Mycology, 43(6): 565-569.

Hakim, J. G., Gangaidzo, I. T., Heyderman, R. S., Mielke, J., Mushangi, E., Taziwa, A., Robertson,V.J, Musvaire, P and Mason, P. R. (2000). Impact of HIV infection on meningitis in Harare, Zimbabwe: a prospective study of 406 predominantly adult patients. *AIDS (London, England)*, 14(10): 1401-1407.

Haynes, B. F., Pantaleo, G., and Fauci, A. S. (1996). Toward an understanding of the correlates of protective immunity to HIV infection. *Science*, 271(5247): 324-328.

Jain, N., Wickes, B. L., Keller, S. M., Fu, J., Casadevall, A., Jain, P., Ragan, M.A., Banerjee, U. and Fries, B. C. (2005). Molecular epidemiology of clinical *Cryptococcus neoformans* strains from India. *Journal of Clinical Microbiology*, 43(11): 5733-5742.

Janocha-litwin, J., and Simon, K. (2012). Recurrent meningitis – A review of current literature Department of Infectious Disease , Provincial Hospital in Wrocław, 67(1): 41-45.

Kambugu, Meya, Meya, D.B, Rhein, J., O'Brien, M., Janoff, E.N., Ronald, A.R., Kamya, M.R. and Boulware. (2008). Outcomes of cryptococcal meningitis in Uganda before and after the availability of highly active antiretroviral therapy. *Clin Infect Dis.*, 46: 1694-1701.

Katwere, M., Kambugu, A., Piloya, T., Wong, M., Hendel-Paterson, B., Sande, M. A, Ronald, A., and Colebunders, R. (2009). Clinical presentation and aetiologies of acute or complicated headache among HIV-seropositive patients in a Ugandan clinic. *Journal of the International AIDS Society*, 12(1): 21.

Kelley, C. F., Kitchen, C. M. R., Hunt, P. W., Rodriguez, B., Hecht, F. M., Kitahata, M., Crane, H.M., and Deeks, S. G. (2009). Incomplete Peripheral CD4 + Cell Count Restoration in

HIV-Infected Patients Receiving Long-Term Antiretroviral Treatment, *Clinical Infectious Diseases*, 48(6): 787-794.

Kennedy, D. H., and Fallón, R. J. (1979). Tuberculous meningitis. *Jama*, 241(3): 264-268.

Kisenge, P. R., Hawkins, A. T., Maro, V. P., Mchele, J. P. D., Swai, N. S., Mueller, A., and Houpt, E. R. (2007). *BMC Infectious Diseases*, 5: 1-5.

Kozel, T. R., and Bauman, S. K. (2012). CrAg Lateral Flow Assay for Cryptococcosis. *Expert Opin Med Diagn.*, 6(3): 2-3.

Kozubowski, L., and Heitman, J. (2012). Profiling a killer, the development of *Cryptococcus neoformans*. *FEMS Microbiology Reviews*, 36(1): 78-94.

Kronstad, J., Saikia, S., Nielson, E. D., Kretschmer, M., Jung, W., Hu, G., Geddes, M. H. J., Griffiths, J. M., Choi, E. J., Cadieux, B. and Caza, M. (2012). Adaptation of Cryptococcus neoformans to mammalian hosts: integrated regulation of metabolism and virulence. *Eukaryotic cell*, 11(2): 109-118.

Kumar, R. (2005). Aseptic meningitis: diagnosis and management. *The Indian Journal of Pediatrics*, *72*(1): 57-63.

Kumar, S., Wanchu, A., Chakrabarti, A., Sharma, A., Bambery, P., and Singh, S. (2008). Cryptococcal meningitis in HIV infected: Experience from a North Indian tertiary center. *Neurology India*, 56(4): 444-449.

La Hoz, R. M., and Pappas, P. G. (2013). Cryptococcal infections: Changing epidemiology and implications for therapy. *Drugs*, 73(6): 495-504.

Larsen, R. A., Bauer, M., Thomas, A. M., and Graybill, J. R. (2004). Amphotericin B and Fluconazole, a Potent Combination Therapy for Cryptococcal Meningitis. *Antimicrobial Agents and Chemotherapy*, 48(3): 985-991.

Lartey, M., Essel, A., Kenu, E., Ganu, V., and Neequaye, A. (2015). Causes of death in hospitalized HIV patients in the ear ly anti-retroviral therapy era, 49(1): 7-11.

Lee, S. J., Choi, H. K., Son, J., Kim, K. H., and Lee, S. H. (2011). Cryptococcal meningitis in patients with or without human immunodeficiency virus: Experience in a tertiary hospital. *Yonsei Medical Journal*, 52(3): 482-487.

Lessells, R. J., Mutevedzi, P. C., Heller, T., and Newell, M. L. (2011). Poor long-term outcomes for cryptococcal meningitis in rural South Africa. *S Afr Med J*, 101(4): 251-252.

Levitz, S. M. (1991). The ecology of *Cryptococcus neoformans* and the epidemiology of cryptococcosis. *Review of Infectious Diseases*, 13(6): 1163-1169.

Lieb, G., Krauss, J., Collmann, H., Schrod, L., and Sörensen, N. (1996). Recurrent bacterial meningitis. *European journal of pediatrics*, 155(1): 26-30.

Likasitwattanakul, S., Poneprasert, B., and Sirisanthana, V. (1999). Cryptococcosis in HIV-infected children: 935-939.

Lortholary, O., Poizat, G., Zeller, V., Neuville, S., Boibieux, A., Alvarez, M., Dellamonica, P. and Chêne, G. (2006). Long-term outcome of AIDS-associated cryptococcosis in the era of combination antiretroviral therapy. *AIDS (London, England)*, 20(17): 2183-2191.

MacDougall, L., Kidd, S. E., Galanis, E., Mak, S., Leslie, M. J., Cieslak, P. R., James W. Kronstad, Muhammad G. Morshed, and Bartlett, K. H. (2007). Spread of *Cryptococcus*

gattii in British Columbia, Canada, and detection in the Pacific Northwest, USA. *Emerging infectious diseases*, 13(1): 42.

Machado, L. D. R., Livramento, J. A., and Vianna, L. S. (2013). Cerebrospinal fluid analysis in infectious diseases of the nervous system: when to ask, what to ask, what to expect. *Arquivos de Neuro-Psiquiatria*, 71(9B): 693-698.

Maleeha, A., Rubeena, H., and Tahir, M. (2006). Bacterial meningitis: A diagnostic approach Biomedica. *Bio*-13 (A): 22.

Marshall, W.J. and S. K. Bangert, S.K. (2008). *Clinical Biochemistry: Metabolic and Clinical aspects*. Elsevier Health Sciences: 683.

Mathiesen, I. H. M., Knudsen, J. D., Gerstoft, J., Cowan, S., and Benfield, T. (2012). Outcome of HIV-1-associated cryptococcal meningitis, Denmark 1988–2008. *Scandinavian Journal of Infectious Diseases*, 44(3): 197-200.

McCarthy, K. M., Morgan, J., Wannemuehler, K. A., Mirza, S. A., Gould, S. M., Mhlongo, N., Moeng, P., Maloba, B.R., Crewe-Brown, H.H., Brandt, M.E. and Hajjeh, R. A. (2006). Population-based surveillance for cryptococcosis in an antiretroviral-naive South African province with a high HIV seroprevalence. *Aids*, 20(17): 2199-2206.

Mdodo, R., Brown, K., Omonge, E., Jaoko, W., Baddley, J., Pappas, P., Colette-Kempf, M., and Jolly, P. E. (2010). The prevalence, clinical features, risk factors and outcome associated with cryptococcal meningitis in HIV positive patients in Kenya. *East African Medical Journal*, 87(12): 481-487.

Mirza, S. A., Phelan, M., Rimland, D., Graviss, E., Hamill, R., Brandt, M. E., Gardner and Hajjeh, R. A. (2003). The changing epidemiology of cryptococcosis: An update from

population-based active surveillance in 2 large metropolitan areas, 1992-2000. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, 36(6): 789-794.

Mitchell T. G. (2004): Medical mycology. In: Jawetz, Menick and Adelberg's Medical Microbiology, 23rd edition. Brooks G. F., Butel J. S. and Morse S. A. USA. McGraw-Hill: 623-660.

Mitchell, T. G., and Perfect, J. R. (1995). Cryptococcosis in the era of AIDS-100 years after the discovery of *Cryptococcus neoformans*. *Clinical microbiology reviews*, 8(4): 515-548.

Munyazesa, E., Emile, I., Mutimura, E., Hoover, D. R., Shi, Q., Mcginn, A. P., Musiime, S., and Anastos, K. (2012). Assessment of haematological parameters in HIV-infected and uninfected Rwandan women : a cross-sectional study: pp 1-9.

Nester, E. W., Anderson, D. G., C. Evans Roberts, J., and Nester, M. T. (2009). Microbiology:A Human Perspective. WCM Mcgraw-Hill: New York: 666-669.

Obirikorang, C., and Yeboah, F. A. (2009). Blood haemoglobin measurement as a predictive indicator for the progression of HIV/AIDS in resource-limited setting. *J Biomed Sci*, 16(102): 10-1186.

Olivares, L. R. C., Martínez, K. M., Cruz, R. M. B., Rivera, M. A. M., Meyer, W., Espinosa, R. A. A., Martinez R.L., and Santos, G. M. R. P. Y. (2009). Genotyping of Mexican *Cryptococcus neoformans* and *C. gattii* isolates by PCR-fingerprinting. *Medical Mycology : Official Publication of the International Society for Human and Animal Mycology*, 47(7): 713-721.

Ooi, M. H., Wong, S. C., Lewthwaite, P., Cardosa, M. J., & Solomon, T. (2010). Clinical features, diagnosis, and management of enterovirus 71. *The Lancet Neurology*, 9(11): 1097-1105.

Othman, N., Atiqah, N., Abdullah, N., and Wahab, Z. A. (2004). Cryptococcal Meningitis in an Immunocompetent Child : A Case Report and Literature Review. *Health (San Francisco)*, 35(4); 930-934.

Owusu, M., Nguah, S. B., Boaitey, Y. A., Badu-Boateng, E., Abubakr, A.-R., Lartey, R. A., and Adu-Sarkodie, Y. (2012). Aetiological agents of cerebrospinal meningitis: a retrospective study from a teaching hospital in Ghana. *Annals of Clinical Microbiology and Antimicrobials*, 11(1): 28.

Park, B. J., Wannemuehler, K. A., Marston, B. J., Govender, N., Pappas, P. G., and Chiller, T. M. (2009). Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. *AIDS (London, England)*, 23(4): 525-530.

Patil, R. T., Sangwan, J., Juyal, D., and Lathwal, S. (2013). Meningitis due to *Cryptococcus gattii* in an immunocompetent patient. *Journal of Clinical and Diagnostic Research*, 7(10): 2274-2275.

Perfect, J. R., and Casadevall, A. (2002). Cryptococcosis. *Infect Dis Clin North Am*, 16(4): 837-74, v–vi.

Perfect, J. R., Dismukes, W. E., Dromer, F., Goldman, D. L., Graybill, J. R., Hamill, R. J., Harrison, T.S. and Sorrell, T. C. (2010). Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the Infectious Diseases Society of America. *Clinical*

Infectious Diseases : An Official Publication of the Infectious Diseases Society of America, 50(3): 291-322.

Powderly, W. G. (1993). Cryptococcal meningitis and AIDS. *Clinical Infectious Diseases*, 17(5), 837-842.

Price, R. W. (1996). Neurological complications of HIV infection. *The Lancet*, 348(9025): 445-452.

Raman Sharma, R. (2010). Fungal infections of the nervous system: current perspective and controversies in management. *Int J Surg*, 8: 591-601.

Ramos, C. L., Fonseca, F. L., Rodrigues, J., Guimarães, A. J., Cinelli, L. P., Miranda, K., Leonardo Nimrichter, Arturo Casadevall, Luiz R. Travassos Rodrigues, M. L. (2012). Chitin-like molecules associate with Cryptococcus neoformans glucuronoxylomannan to form a glycan complex with previously unknown properties. *Eukaryotic cell*, 11(9), 1086-1094.

Reid, H., and Fallon., R. J. (1992). Bacterial infections. *Green Field's Neuropathology. Oxford University Press, New York*: 302-334.

Roos and Brosch, 2012, Meningitis and Encephalitis. In McKean, S.C, Ross, J. J, Dressler, D.D, Brotman, D. J., and Ginseng, J. S., ed. Principles and Practice of Hospital Medicine. McGraw-Hill: New-York: 1-7

Rosario, I., Hermoso De Mendoza, M., Déniz, S., Soro, G., Álamo, I., and Acosta, B. (2005). Isolation of *Cryptococcus species* including *C. neoformans* from cloaca of pigeons. *Mycoses*, 48(6): 421-424.

Rotbart, H. A. (1997). Viral meningitis and the aseptic meningitis syndrome. *Infections of the central nervous system. Philadelphia: Lippincot-Raven Publishers*: 239-63.

Rotbart, H. A. (1999, December). Viral meningitis. In *Seminars in neurology* (Vol. 20, No. 3: pp. 277-292).

Rozenbaum, R., and Gonçalves, A. J. (1994). Clinical epidemiological study of 171 cases of cryptococcosis. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, 18(3): 369-380.

Saag, M. S., Cloud, G. A., Graybill, J. R., Sobel, J. D., Tuazon, C. U., Johnson, P. C., Fessel, W.J., and Dismukes, W. E. (1999). A comparison of itraconazole versus fluconazole as maintenance therapy for AIDS-associated cryptococcal meningitis. *Clinical infectious diseases*, 28(2): 291-296.

Saag, M. S., Graybill, R. J., Larsen, R. A, Pappas, P. G., Perfect, J. R., Powderly, W. G., Sobel, J.D and Dismukes, W. E. (2000). Practice guidelines for the management of cryptococcal disease. Infectious Diseases Society of America. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, 30(4): 710-718.

Saag, M. S., Powderly, W. G., Cloud, G. A., Robinson, P., Grieco, M. H., Sharkey, P. K., Thompson, S.E., and Dismukes, W. E. (1992). Comparison of amphotericin B with fluconazole in the treatment of acute AIDS-associated cryptococcal meningitis. *New England Journal of Medicine*, 326(2): 83-89.

Sabetta, J. R., and Andriole, V. T. (1985). Cryptococcal infection of the central nervous system. *The Medical clinics of North America*, 69(2): 333-344.

Saha, D. C., Xess, I., and Jain, N. (2008). Evaluation of conventional & serological methods for rapid diagnosis of cryptococcosis. *Indian Journal of Medical Research*, 127(5): 483-488.

Sarosi, G. A., Parker, J. D., Doto, I. L., and Tosh, F. E. (1969). Amphotericin B in cryptococcal meningitis: long-term results of treatment. *Annals of internal medicine*, 71(6): 1079-1087.

Schaars, C. F., Meintjes, G. A., Morroni, C., Post, F. A., and Maartens, G. (2006). Outcome of AIDS-associated cryptococcal meningitis initially treated with 200 mg/day or 400 mg/day of fluconazole. *BMC Infectious Diseases*, 6: 118.

Schmidt S., Reiter-Owona, I., Hotz M., Mewes, J., and Biniek, R. (1995). An unusual case of central nervous system cryptococcosis. *ClinNeurol Neurosurg*, 97(1): 23-27.

Sellebjerg, F., and Tumani, H. (2009). Routine c erebrospinal fluid (CSF) analysis: pp. 5-17.

Smith, E. M., and Samadian, S. (1993). Use of the erythrocyte sedimentation rate in the elderly. *British journal of hospital medicine*, 51(8): 394-397.

Snow, R. M., and Dismukes, W. E. (1975). Cryptococcal meningitis: diagnostic value of cryptococcal antigen in cerebrospinal fluid. *Archives of internal medicine*, 135(9): 1155-1157.

Spanos, A., Harrell, F. E., and Durack, D. T. (1989). Differential diagnosis of acute meningitis: an analysis of the predictive value of initial observations. *Jama*, 262(19): 2700-2707.

Speed, B., and Dunt, D. (1995). Clinical and host differences between infections with the two varieties of *Cryptococcus neoformans*. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, 21(1): 28-34.

Tagoe, D.N.A., and Asantewaa, E., (2011). Scholars research library Profiling Haematological Changes in HIV Patients Attending Fevers Clinic at the Central Regional Hospital in Cape Coast , Ghana : A Case-Control Study." *Archives of Applied Science Research* 3(5): 326-331.

Tunlayadechanont, S., Viranuvatti, K., Phuapradit, P., Sathapatayavong, B., Tantirittisak, T., and Boongird, P. (1997). Cryptococccal meningitis in patients with non-HIV and HIV infection.*Neurol J Southeast Asia,* 2: 45-50.

UNAIDS, U. (2013). AIDS epidemic update: November 2009.

Van der Horst, C. M., Saag, M. S., Cloud, G. A., Hamill, R. J., Graybill, J. R., Sobel, J. D., Johnson, P.C., and Dismukes, W. E. (1997). Treatment of cryptococcal meningitis associated with the acquired immunodeficiency syndrome. *New England Journal of Medicine*, 337(1): 15-21.

Vengerov, I. I. (1979). Laboratory diagnosis of meningitis. *Meditsinskaia Sestra,* 38(4): 45-47.

White, D. N., and Fyles, S. (1950). Cerebrospinal Fluid. *Canadian Medical Association Journal*, 62(4): 384-386.

WHO Rapid advice (2011). Diagnosis, prevention and management of cryptococcal disease in HIV-infected adults, adolescents and children. Geneva: World Health Organization.

Wu, T. C., and Koo, S. Y. (1983). Comparison of three commercial cryptococcal latex kits for detection of cryptococcal antigen. *Journal of clinical microbiology*, 18(5): 1127-1130.

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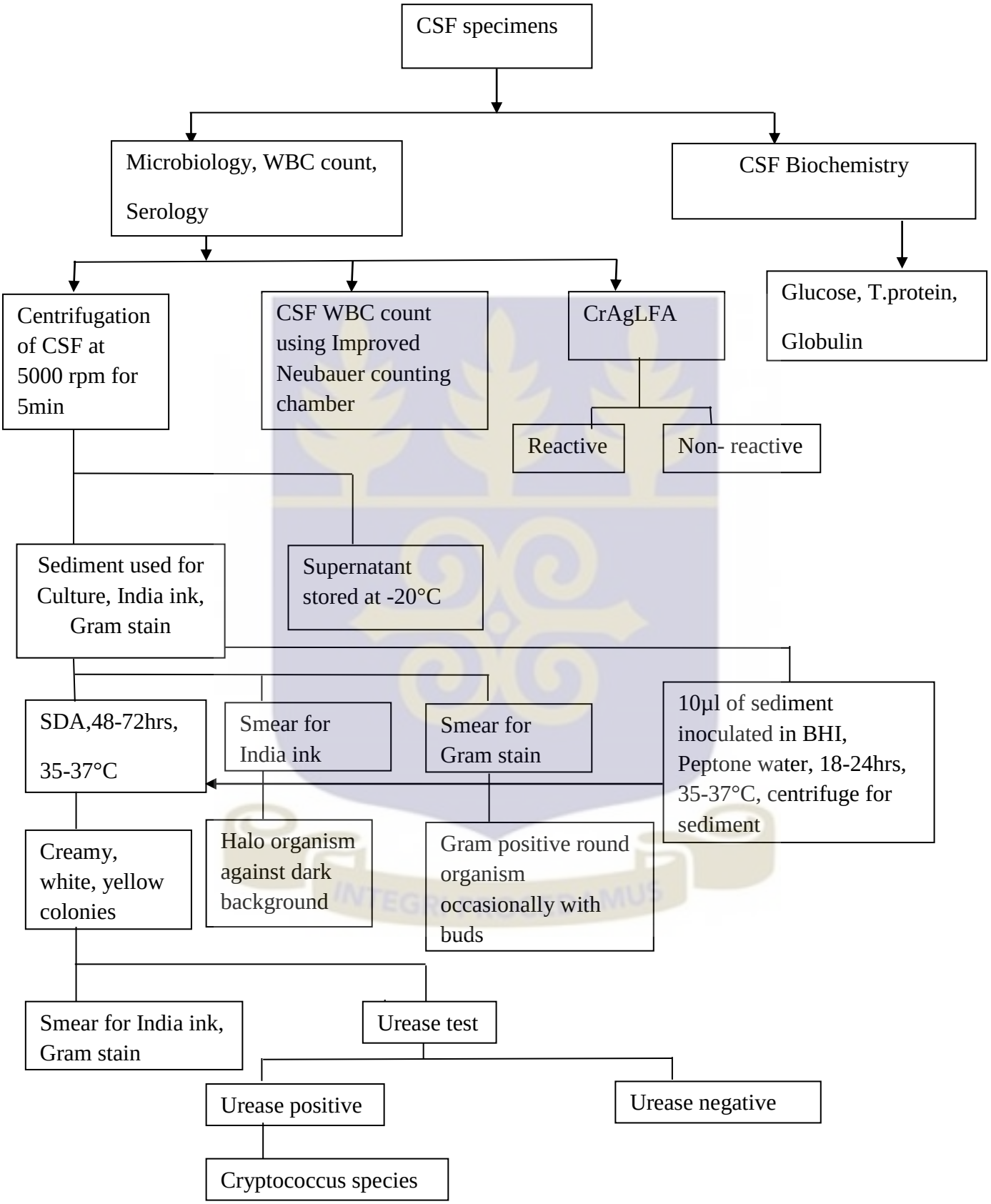
Xu C.Y, Zhu H. M, Wu J. H, Wen H, and Liu, C.J. (2014). Increased permeability of blood-brain barrier is mediated by serine protease during Cryptococcus meningitis. *J Int Med Res.*, 42(1): 85-92.

Xu, J., and Mitchell, T. G. (2003). Comparative gene genealogical analyses of strains of serotype AD identify recombination in populations of serotypes A and D in the human pathogenic yeast *Cryptococcus neoformans*. *Microbiology*, 149(8): 2147-2154.

APPENDIX 1: FLOWCHART OF LABORATORY PROCEDURE



APPENDIX II: INFORMATION AND CONSENT FORM

INFORMATION SHEET

Title: [*Cryptococcal meningitis in hospitalized HIV patients at the Fever's Unit, Korle-Bu*]

Principal Investigator: [Japheth A. Opintan]

Address: [Department of Microbiology, University of Ghana Medical School, College of Health Sciences, P.O. Box 4236, Accra, Ghana]

General information about the research

Cryptococcal meningitis (CM) is a serious fungal infection that usually affects people with advanced HIV/AIDS. The disease is not contagious, meaning it cannot spread from person-to-person. CM specifically occurs after the fungus, *Cryptococcus* has been inhaled from the environment, and spread from the lungs to the brain, causing meningitis. In healthy people, the fungus usually does not cause serious illness because the immune system can fight off the infection. Symptoms of CM include headache, fever, neck pain, and altered mental status. Body tissues such as cerebrospinal fluid (CSF) and blood are required to laboratory confirm the disease. When the disease is diagnosed early enough, appropriate therapy can be given to prevent fatal consequences such as death. We wish to work with you to find the prevalence and outcome of CM among hospitalized HIV patients. This would ensure that HIV patients who develop CM or who are at the advanced stages of HIV/AID are managed well to prevent death.

Study procedure

All patients presenting with symptoms suggestive of cryptococcal meningitis at the Fever's Unit will be eligible for the study. The symptoms include headaches, seizures, cranial nerve palsies, altered mental state-confusion, delirium, inappropriate speech, and coma among others. Patient

will be admitted to the ward and informed consent sought from the patient if the mental state is normal or from a caregiver or next of kin if mental state is abnormal but patient meets inclusion criteria.

History of illness and physical examination findings will be documented. Standard of care laboratory investigations will be performed. These include a complete blood count, blood urea and electrolytes, CD4 count, HIV Viral loads, CT scan of the brain where affordable. CSF samples will be taken by lumbar puncture (when intra cranial pressures is not high) under aseptic conditions by a medical officer. A total of about 4 ml CSF will be collected for various laboratory investigations including chemistries, microscopy, serology and microbiological culture. About 3 ml venous blood will also be taken for other laboratory investigations.

As part of standard of care, prompt treatment will be initiated after specimens for the laboratory investigations have taken. This will include treatment for cerebral toxoplasmosis, bacterial meningitis, tuberculous meningitis or cryptococcal meningitis. Appropriate anti-infectives will be adapted on receipt of results.

Possible Risks and Discomforts

The procedure for obtaining samples for laboratory tests can be associated with very rare risks including mild pain, bruising, bleeding or infection. Aseptical procedures will be used, and a qualified medical doctor will take lumbar puncture under anesthesia. CSF and blood loss for the study will not adversely affect your health. Any discomfort will be immediately evaluated and managed by medical officers.

Possible Benefits

As a patient you will benefit from a calculated effort at identifying causative organisms in the CSF. This should result in an improvement in your management. The CSF laboratory investigations will be analyzed at no cost to you.

Confidentiality

All the information collected in the study will be kept confidentially in a secure way. Results of investigations or other information that we collect will only be shared with the medical staff taking care of you and authorized members of the research team. Any data that may be published will not reveal your identity.

Compensation

There will be no cost or payment to you for participation in this study.

Freedom to refuse or withdraw

You should only join the study if you want to. You can ask as many questions as you like. You can leave the study at any time without giving any reason and this will not affect your medical care and treatment provided.

Contacts for Additional Information

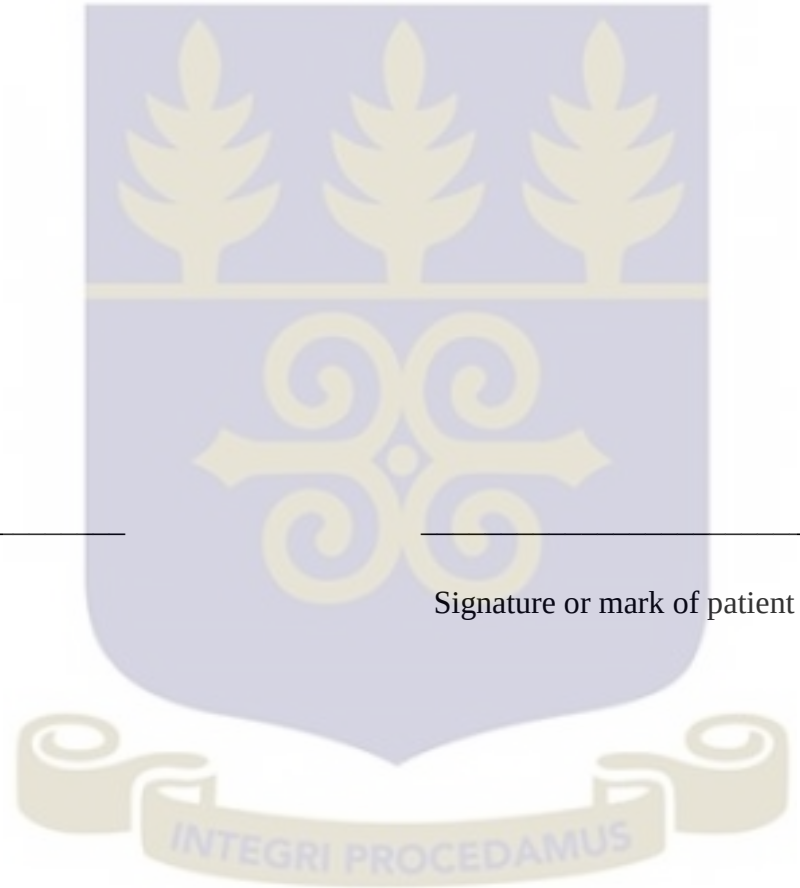
If you have any questions or concerns, please contact Dr. Japheth A. Opintan (0244789209), Benedict K. Awadzi (0246472587) at the Microbiology Department, UGMS (now SBAHS) or Dr. Ernest Kenu (0244592122), at the Fever's Unit, Korle-Bu.

Your rights as a Participant

This research has been reviewed and approved by the ethical and protocol review committee, University of Ghana Medical School. If you have any questions about your rights as a research participant you can contact the research office through: Telephone +233 302672029, E-mail research@ugms.edu.gh, P.O. Box GP 4236, Korle-Bu, Accra-Ghana

CONSENT FORM

The above document describing the benefits, risks and procedures for the research title "*Cryptococcal meningitis in hospitalized HIV patients at the Fevers' Unit, Korle-Bu"* has been read and explained to me/caregiver or next of kin. I/caregiver have read and understood the above information. I/caregiver have been given opportunity to ask questions about the research, and have received satisfactory answers. I/caregiver agree to participate in this study as a volunteer.



_____ _____

Date Signature or mark of patient

_____ _____

Date Signature or mark of caregiver

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

_____ _____

Date Signature of Researcher

APPENDIX III: QUESTIONANNAIRE

Study number_____

Abstractor's initials_____

Date of abstraction___/____/___

I. Patient Information

1) Coded ID
_____/_____

2) Gender

❒ 1 – Male

❒ 2 – Female

3) Date of birth

--------/-------/---------- (dd/mm/yy)

_____ years

4) Weight

_____ kg

5) Height

_____ cm

6) Last year of education completed?

❒ 1 – None

❒ 2 – Primary

❒ 3 – JSS

❒ 4 – Middle School

❒ 5 – Sec/Tech

❒ 6 – Tertiary

❒ 9 – Unknown

7) Employed?

❒ 1 – Yes

❒ 2 – No

8) Married?

❒ 1 – Yes

❒ 2 – No

II. Previous history of hospitalization

9) Hospitalized within the last 6 months?

❒ 1 – Yes

❒ 2 – No

10) HIV status

❒ 1 – Positive

❒ 2 – Negative

❒ 3 – Unknown

11) Date of HIV diagnosis?

--------/-------/---------- (dd/mm/yy)

12) Type of HIV

❒ 1 – HIV 1

❒ 2 – HIV 2

❒ 3 – HIV 1 & 2

13) Mode of HIV acquisition?

❒ 1 – Heterosexual

❒ 2 – Homosexual

❒ 3 – Injection drug use

❒ 4 – Transfusion

❒ 5 – Unknown

14) Serial CD4 count starting with most recent test?

❒ 1 – _____ mm/µl

Date: _____/_____/_____

❒ 2 – _____ mm/µl

Date: _____/_____/_____

15) Most recent CD4/CD8 ratio

_____

III. Information on ART therapy

16) Is the patient currently on ART therapy?

❒ 1 – Yes

❒ 2 – No

❒ 3 – Unknown

17) What is the current staging?

❒ 1 – Stage I

❒ 2 – Stage II

❒ 3 – Stage III

18) ART compliance during the last two appointments?

❒ 1 – Yes

❒ 2 – No

❒ 3 – Unknown

IV Clinical Information

19) Date of current hospitalization?

--------/-------/---------- (dd/mm/yy)

20) Clinical symptoms and signs at hospitalization?
(check all that apply please)

❒ 1 – Headache

❒ 2 – Fever

❒ 3 – Nausea/vomiting

❒ 4 – Seizures

❒ 5 – Stiff neck

❒ 6 – Confusion/neurological symptoms

❒ 7 – Others (please state)

_____

_____

21) Duration of current symptoms?

❒ 1 – between 0 to 7 days

❒ 2 – between 8 to 14 days

❒ 3 – between 15 to 21 days

❒ 4 – greater than 21 days

❒ 5 – unknown

V. Physical examination

22) Axillary temperature _____

23) Oral thrush present?

❒ 1 – Yes

❒ 2 – No

24) Meningismus?

❒ 1 – Yes

❒ 2 – No

25) Tachycardia?

❒ 1 – Yes

❒ 2 – No

VI. Intracranial pressure

26) Opening _____ mmHg

27) Closing _____ mmHg

28) Glasgow coma scale _____

Laboratory investigations

VI Haematological

29) Leucocytes _____

30) Lymphocytes _____

31) Haemoglobin _____

32) Platelets _____

33) ESR _____

34) RBS _____

VII. CSF chemistry

35) Protein _____g/L

36) Glucose _____mmol/L

37) WBC count _____

38) Differential _____

VIII Microbiological

39) Gram stain

❒ 1 – G +ve spherical/capsulated cells

❒ 2 – G +ve oval cells

❒ 3 – G +ve diplococci

❒ 4 – G +ve bacteria

40) India Ink test

❒ 1 – Positive

❒ 2 – Negative

41) CSF antigen test

❒ 1 – Positive

❒ 2 – Negative

42) Culture results for Yeast

❒ 1 – Positive

❒ 2 – Negative

43) Organism isolated? Please list

_____

_____

44) Cryptococcus species?

_____

45) Anti-fungal susceptibility

Fluconazole_____ µg/ml

Itraconazole_____ µg/ml

Flucytosine _____ µg/ml

IV. Management during hospitalization

46) Initial therapy given

❒ 1 – IV fluconazole

❒ 2 – oral fluconazole

❒ 3 – IV Penicillin

❒ 4 – Oral penicillin

❒ 5 – Others (please state)

_____

_____

IV. Management for OPD cases

47) Initial therapy given

❒ 1 – Oral fluconazole

❒ 2 – Oral Penicillin

❒ 5 – Others (please state)

_____

_____

48) What was the duration of therapy?

❒ 1 – One week

❒ 2 – Two weeks

❒ 3 – Three weeks

49) Was initial therapy changed/modified in the course of treatment?

❒ 1 – Yes

❒ 2 – No

50) Was CSF sterilized (10-14 days) after therapy?

❒ 1 – Yes

❒ 2 – No

❒ 3 – Unknown

51) Outcome?

❒ 1 – Discharged

❒ 2 – Died

52) Date of discharge/death

--------/-------/---------- (dd/mm/yy)

53) Duration of stay on ward

_____

APPENDIX IV: STUDY REQUEST FORM

Study ID_____

CM STUDY REQUEST FORM

Family Name:…… Date:……

First Name:…… Age:… Sex:….....

Folder #:…… Ward:……...

***Please tick appropriate tests*:**

CSF CULTURE & SEROLOGY ☐

CSF BIOCHEMISTRY CSF ☐

VIRAL LOAD ☐

Doctor's initial:……

APPENDIX V: STUDY LABORATORY REPORT FORM

Patient Folder#_____

MICROBIOLOGICAL RESULTS - CM STUDY

Family Name:…… Date:……..……

First Name:…… Age:…… Sex:…...

Study ID :…….. Ward:…….

***RESULTS*:**

CrAg Test:_____ **Titre**:_____

CSF Appearance:_____ **WBC count**:_____

Gram:_____ **India ink test**:_____

Culture:_____

Susceptibility:

Signature:…….

APPENDIX VI: PREPARATION OF AGAR MEDIA AND REAGENTS

a) Sabouraud dextrose agar (per litre)

i. Composition

65 g Sabouraud dextrose agar powder

10.0 g Mycological peptone

40.0 g Glucose

15.0 g Agar

PH 5.6+0.2 at 25˚C

ii. Preparation

According to the manufacturers' protocol, 500 ml of Sabouraud's dextrose agar (SDA) (OXOID LTD, Lot no. 1419386) was prepared by dissolving 32.5 g of SDA into 500 ml of distilled water. The solution was brought to boil to dissolve powder completely. Solution was then sterilised by autoclaving at 121˚C for 15 minutes. About 25 ml of molten agar was dispensed into each one of disposable Petri dishes. The molten agars in the petri dishes were allowed to solidify at room temperature. The plates were packaged and stored at 2˚C to 8˚C in a fridge. For every batch of SDA plates prepared, one plate was incubated at 37˚C and another plate was streaked with a known *Cryptococcus* spp. for sterility and quality control respectively

d) Preparation of 70% ethanol

One litre of 70% ethanol was prepared by adding 300 ml of distilled water to 700 ml of absolute alcohol. Solution was transferred into a container and labelled with date of preparation and percentage of solution.

d) Sterilization of consumables

Bijoux bottles were slightly opened and autoclaved at 121˚C for 15 minutes. They were then transferred into the hot air oven to dry. The dry Bijoux bottles were transferred to the Fevers' unit for CSF collection.

Eppendolf tubes were fully opened and placed in a clean conical flask and the mouth of it sealed with a foil. They were then autoclaved at 121˚C for 15 minutes. They were then transferred into the hot air oven to dry.

Yellow micropipette tips were arranged in their racks and were autoclaved at 121˚C for 15 minutes. They were then transferred into the hot air oven to dry.

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My Ref. No: MS-AA/C.2/Vol.18[A]

Your Ref. No.

P O Box 4236
Accra
Ghana

18th June, 2014

Dr. Japheth A. Opintan
Department of Microbiology
U. G. M. S.
Korle-Bu

ETHICAL CLEARANCE

Protocol Identification Number: MS-Et/M.11 – P 3.2/2013-2014

The Ethical and Protocol Review Committee of the University of Ghana Medical School on 17th June, 2014 unanimously approved your research proposal.

TITLE OF PROTOCOL: **"Cryptococcal Meningitis in Hospitalized HIV Patients at the Fevers' Unit, Korle-Bu Teaching Hospital"**

PRINCIPAL INVESTIGATOR: **Dr. Japheth A. Opintan**

This approval requires that you submit six-monthly review reports of the protocol to the Committee and a final full review to the Ethical and Protocol Review Committee at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study during and after implementation.

Please note that any significant modification of this project must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the Ethical and Protocol Review Committee within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

This ethical clearance is valid till May, 2016. However, this is subject to the presentation of an annual report yearly whereupon a renewal of the approval will be given.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed: .....
PROFESSOR JENNIFER WELBECK
(CHAIRPERSON, ETHICAL AND PROTOCOL REVIEW COMMITTEE)

cc: Ag. Dean
Head of Department
Research Office