SEROPREVALENCE OF CRYPTOCCOCAL ANTIGENEMIA IN HIV POSITIVE ADULT INPATIENTS WITH SEVERE IMMUNOSUPPRESION ATTENDING THE KENYATTA NATIONAL AND MBAGATHI HOSPITALS

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I certify that this dissertation is my original work and has not been submitted for a degree to any other University.

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DEDICATION

This book is dedicated to my loving parents Josephine and David Muchiri, thank you for everything, I love you and God bless you.

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The Almighty God, You are Faithful

My supervisors for your honest guidance, patience and expertise during this study

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TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

TABLES

FIGURES

LIST OF ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ART	Anti-Retroviral Treatment
ASSURED	Affordable, Sensitive, Specific, User friendly, Robust, Rapid and Equipment free
CCC	Comprehensive Care Clinic
CSF	Cerebral Spinal Fluid
CI	Confidence Interval
CM	Cryptococcal Meningitis
CRAG/CrAg	Cryptococcal antigen
EIA	Enzyme Immuno Assay
ELISA	Enzyme-Linked Immunosorbent Assay
GXM	Glucuronoxylomannan
HAART	Highly Active Antiretroviral Agents
HIV	Human Immunodeficiency Virus
HR	Hazard Ratio
IRIS	Immune Reconstitution Syndrome
KAIS	Kenya AIDS Indicator Survey
KDHS	Kenya Demographic Health Survey
KNH	Kenyatta National Hospital
LA	Latex Agglutination
LFA	Lateral Flow Assay

OR	Odds Ratio
PLWHA	People Living With HIV/AIDS
PI	Principle Investigator
RR	Relative Risk
SPSS	Statistical Package for the Social Sciences
SSA	Sub Saharan Africa
TB	Tuberculosis
WHO	World Health Organization
UNAIDS	United Nations AIDS

ABSTRACT

Background

In the world and especially Sub Saharan Africa (SSA), HIV is a leading public health concern. Cryptococcal meningitis (C.M) is the 2nd leading opportunistic infection and is an AIDS defining illness associated with high morbidity and mortality despite the use of HAART and effective anti-fungal treatment. Screening for cryptococcal antigenemia with the use of pre-emptive antifungal therapy may reduce the burden of disease in populations with a high prevalence of cryptococcal antigenemia. The prevalence of cryptococcal antigenemia in Nairobi Kenya has not been established.

Objectives

To determine the prevalence of cryptococcal antigenemia and associated factors in HIV-infected adult in-patients HAART naïve with low CD4 counts, at the KNH and Mbagathi hospitals.

Study design and settings

Cross sectional descriptive study that was carried out at Kenyatta National and Mbagathi hospitals' in-patient medical wards.

Methods

A total of 196 HIV ELISA positive adult patients HAARTnaïve, with a CD4 of ≤100 cells/µl admitted to the medical wards were consecutively recruited. Their demographic data was captured using a pretested structured questionnaire and were clinically evaluated for WHO clinical staging and symptoms or signs of meningitis. All participants had a serum CRAG assessment and CD4 count done using lateral flow assay, an ELISA method and CyFlow respectively. Prevalence of CRAG serum positivity was ascertained. Association of sero-positivity to the demographic, laboratory and clinical characteristics was determined using Chi-square and Student's T-test. P Value of less than 0.05 was considered significant.

Results

The seroprevalence of cryptococcal antigenemia was 13.8%. On bivariate analysis cryptococcal antigenemia was associated with neck stiffness. OR 3.9 (1.4-10.7, 95% CI)

Seropositivity was not related to other demographic and clinical factors.

Conclusion

The seroprevalence of cryptococcal antigenemia is high in this population.

1.0 LITERATURE REVIEW

1.1 BURDEN OF HIV AND CRYPTOCOCCAL DISEASE

The Human Immunodeficiency Virus (HIV) pandemic is one of the leading health challenges in the world today. Worldwide as reported in the United Nation Acquired Immunodeficiency Syndrome (UNAIDS 2011 world AIDS day), it is estimated that 34 million people globally are living with HIV. In the year 2010 there were 2.7 million [2.4 – 2.9 million] new HIV infections and 1.8 million [1.6– 1.9 million] AIDS-related deaths. Sub Saharan Africa carries the largest burden as 22 million people living with HIV are in this part of the world. This accounts for two thirds (67%) of all people living with HIV, and the region also accounted for three quarters (75%) of AIDS deaths.[1]

In Kenya as reported by the Kenya Health Demographic survey (KDHS) 2010, the prevalence among adults aged 15-49 years, is estimated at 6%. The national HIV prevalence of 6.4% (6.1-

6.9%) among adults aged 15-49 years is much higher than the global HIV prevalence of 5%

[2].National Aids Control Council and National AIDS and STD Control Program July 2011, estimates the number of people living with HIV at 1.6 million (1.5-1.7), new adult infections 105,000 (90-125,000) and the annual AIDS death at 65,000 (56-74,000)

Cryptococcal neoformans is a leading opportunistic pathogen and a leading cause of mortality in AIDS patients in the developing world. Cryptoccocal meningitis is the 2nd commonest opportunistic infection in SSA[2] ,with a prevalence of 17-38%[3] . It is the commonest cause of adult meningitis in SSA, and in Malawi it made up 26.5% of all cases of meningitis [4], 39.1% in a case series from Democratic Republic of Congo[5] and 45% in Zimbabwe[6].

Locally, in a retrospective observational study done by Jowi et al at a private hospital in Nairobi Kenya to determine the clinical and laboratory characteristics of hospitalised HIV-infected

patients with neurological complications, a total of 708 patients were recruited and the commonest neurological complication was cryptoccocal meningitis with a prevelance of 22% .[7]

Furthermore, in a cross sectional study done by Langat at Moi Teaching and Refferal Hospital in Eldoret for his MMed dissertation (unpublished) to determine the common causes of adult meningitis amongst patients admitted to the general medical ward, a total of 81 patients were recruited and out of these 34 (42%) had cryptoccoccal meningitis which was shown to be the commonest cause.

Cryptoccocal meningitis is assosiated with a 100% mortality if left untreated, and with optimal treatment in the developed world it has a mortality of 9-38%[8910], while in SSA mortality ranges between 37-58% [1112]. It is a leading cause of death accounting for 20% of all mortality in several cohorts[13] with acute mortality in the range of 24%-43%[1415]. In SSA cryptoccocal meningitis caused > 500,000 deaths/year which may exceed those attributed to tuberculosis[16]

1.2 CRYPTOCOCCUS NEOFORMANS

1.2.1 Ecology

Cryptococcus neoformans is an environmental saprophyte. It is an encapsulated, spherical yeast of which two varieties are recognized, *Cryptococcus neoformans var neoformans* and *Cryptococcus neoformans var gattii*. There are four capsular serotypes A through D and serotype AD. Isolates of *C. neoformans var neoformans* may possess capsular serotypes A, D or AD and isolates of *C. neoformans var gattii* are serotypes B or C.

In addition to their serotypes the two varieties of *C. neoformans* also differ in certain biochemical properties and epidemiology, *C. neoformans var neoformans* is found in soil contaminated with pigeon avian feces while C neoformans var gattii is mainly found in eucalyptus trees.

The etiology of 99% of infections in AIDS patients is due to *Cryptococcal neoformans var neoformans* serotype A.

1.2.2 Diagnosis

Early diagnosis and treatment are key to improving mortality from cryptococcal disease. Healthcare profesionals should have a low threshold for suspecting cryptococcal disease. Diagnosis of cryptoccocal meningitis involves detection of cryptococcus in the CSF. Culture of CSF is considered to be the gold standard for diagnosis however it takes from 3 days to 1 month to grow. Another diagnostic method is by the demonstration of encapsulated yeast in india ink preparation from CSF; though highly specific at 100% it has low sensitivity of 50-80%[17].

Obtaining a reliable CSF sample involves the use of an aseptic technique which in many rural settings is not possible. In addition, laboratory and technical support is required to make a diagnosis of cryptoccocal disease. With a large burden of HIV being in SSA where the majority of patients will be in a rural set up, alternative methods of identifying patients with cryptoccocal disease have been studied.

Antigen detection represents the most immediate and rapid way for diagnosis of cryptococcus, using a sample of CSF, serum or urine. New point-of-care assays for detection of cryptococcal antigen for the use of diagnosis and screening have been studied.

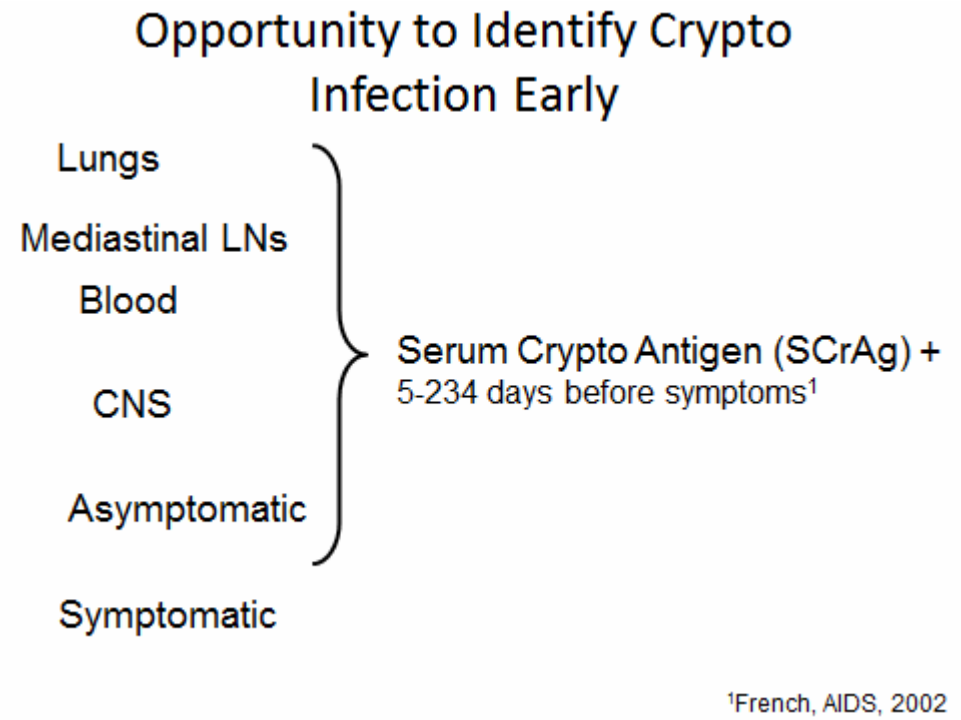
Latex agglutination (L.A), lateral flow assays (L.F.A) and enyme immunoassay (E.I.A) have a high sensitivity and specificity of both serum and CSF at 99% and 83.3% respectively. The assays detect cryptococcal polysacharide capsule glucoronoxylomannan capsule (GXM) with the use of monoclonal antibodies selected to have a broad reactivity acoss the 4 major serotypes of *cryptococcus neoformans*.

EIA is expensive and not feasible in poor resource settings. LFA has several advantages over LA CRAG assay. It is less expensive, has a rapid 5-15 minutes turn around time, requires little training for its use and interpretation, can be performed with minimum labaratory infarstructure and without refrigeration since it is stable at room temperature. It satisfies most of the WHO ASSURED criteria for point of care tests.

The above point of care immunoassays for cryptococcal antigen would greatly facilitate the early diagnosis of patients presenting with symptoms of cryptoccocal meningitis. It can also be used to intervene early to prevent cryptococcal meningitis because CRAG immunoassays are positive before the development of clinical and apparent disease[218] .

1.3 CRYPTOCOCCAL ANTIGENEMIA

A positive serum CRAG indicates extra pulmonary , disseminated and systemic infection especially in AIDS[18] and in some cases progression to severe symptomatic cryptococcosis is inevitable unless appropriate antifungal treatement is given. This is detectable in serum at a median of 5 to 324 days prior to the development of CM[2] . The sub-acute nature of CM allows for effective interventions e.g pre-emptive treatment to prevent the assosiated mortality and morbidity [19] .



CM occurs both before and after initiation of ART. Screening for cryptoccocal disease by the use of serum CRAG especially in patients with advanced immunosuppression can potentially prevent

mortality and morbidity assosiated with unmasking form of immune reconstitution syndrome (IRIS) which accounted for 30% of CM in a study done in Uganda[20].

Screening for cryptoccocal antigenemia is not in the US HIV guidelines due to the geographical variation in the prevelance of cryptoccocosis according to the Infectious Disease Society of America, however in areas of high prevelance of cryptococcal antigenemia, in resource poor settings where it is common-place for patients to present with advanced HIV disease, screening is advocated.

The WHO rapid advice on the diagnosis, prevention and management of cryptococcal disease guidelines 2011 states that routine serum or plasma CrAg screening in ART-naïve adults, followed by pre-emptive anti-fungal therapy if CrAg-positive, to reduce the development of Cryptococcal disease, may be considered prior to ART initiation in patients with a CD4 count less than 100 cells/mm3, where this population has a high prevalence of cryptococcal antigenemia of >3% [21]

1.4 UTILITY OF SERUM CRAG AS A TOOL FOR PREVENTION OF UNMASKED CRYPTOCOCCAL IRIS

IRIS describes a collection of inflammatory disorders associated with paradoxical worsening of preexisting infectious processes following the initiation of highly active antiretroviral therapy (HAART) in HIV-infected individuals[22].Rapid restoration of immune function leads to enhanced cell-mediated response to either live, dead or shed antigen that may present as either unmasking of a latent infection, that may be subclinical or reccurence of symptoms and signs of previously diagnosed and treated infection. Clinical manifestations of cryptococcal IRIS include: meningitis, mediastinal lymphadenitis, cavitatory pneumonia and cryptococcomas, commonest being meningitis.

Currently up to 70% of all cryptococcal cases in some African countries present after the diagnosis of HIV infection is made and approximately 30% present after the initiation of ART[16] [23]

Cryptococcal IRIS has been reported in 6-30% of patients with cryptococcal meningitis following commencement of ART[23 24] . It is more common in SSA because patients present with very advanced disease especially in endemic areas, this could be due to unmasking of the sub-clinical disease therefore screening with serum cryptococcal antigen prior to ART may prevent such cases thereby reducing mortality. Cryptococcal meningitis is among the leading causes of IRIS mortality after tuberculosis immune reconstitution.

The use of serum CRAG test post treatment is poorly characterised as cryptoccocal neoformans possess a capsule of high mollecular weight which results in its slow clearance from serum and CSF and a positive serum CRAG can persist for many years after treatement.

1.5 PREVALENCE OF CRYPTOCOCCAL ANTIGENEMIA IN HIV

Several studies have been done on cryptococcal antigenemia. Locally an out-patient study done by Family AIDS Care and Education Services (FACES) by Kendi and colleges inNyanza Province from November 2009 to September 2010, recruited 1762 patients with CD4 < 100, out of whom 108 patients (6.2%) had cryptococcal antigenemia[25] .

Wanjaga et al conducted an operational study on universal screening of Tanzanian HIV infected adult in-patientswith serum cryptococcal antigen to improve diagnosis and reduce mortality,363 study participants were recruited.A median CD4 count of 209 cell/mm³ was obtained,however 93 participants(27.9%) had a CD4 count of <100 cells/mm³ and the prevalence of cryptococcal antigenemia in this sub-population was found to be 15%[26]

In Uganda a cross-sectional in-patientstudy by Oyella et al to determine the prevalence and factors assosiated with cryptococcal antigenemia among severely immunosuppressed HIV infected adults demonstrated among the 367 participants with a median CD4 count of 23 cell/mm³,69 patients (19 %) had cryptoccocal antigenemia[27] .

Meya et al also in Ugandain an out-patient study determined thecost-effectiveness of serum cryptococcal antigen screening to prevent deaths among HIV-infected persons with a CD4 cell count of <100 cells/mL who start HIV therapy in resource-limited settings demonstrated that

amongst 295 participants with a median baseline CD4 count of 15 cells/ul, 26 participants (8.8%) had cryptoccocal antigenemia. It also demonstrated that screening prevented disease and death in 8% of patients started on ART[19].

In Cambodia, an area endemic for cryptoccoccosis, Micol et al in an in-patient study to determine prevalence, determinants of positivity, and clinical utility of cryptococcal antigenemia in Cambodian HIV-infected patients in 2004 enrolled 327 participants with a mean CD4 count of 24. 59 patients (18.1%) had a cryptoccocal antigenemia[28].

In South Africa, Jarvis et al in 2010 in a retrospective study found that, of 707 participants with a baseline median CD4 count of 97 cells/μL, 46 (7%) had a positive CRAG and in a sub-analysis of patients with a CD4 count of ≤ 100 cells/μL 13% had cryptococcal antigenemia. Cyptoccocal antigenaemia was 100% sensitive for predicting development of cryptococcal meningitis during the first year of ART. Most (92%) cases of cryptococcal meningitis developed in patients with a CD4 count ≤100 cells/μL[14].

1.6 MORTALITY IN CRYPTOCOCCAL ANTIGENEMIA

In a Retrospective study done by Jarvis et al 2010 in South Africa to screen for cryptococcal antigenemia in patients accessing an antiretroviral treatment program in South Africa established that CRAG-positive patients were at far higher risk of mortality than antigen negative patients during the one year follow-up period (HR = 4.75, 95% CI 2.6-8.8, p<0.001). After adjustment for CD4 cell count, viral load, age and sex, baseline cryptococcal antigenemia remained a strong independent risk factor for death (adjusted HR = 3.2; 95% CI 1.5-6.6, p<0.001). This relationship was also found when the analysis was restricted to patients with no prior history of cryptococcal disease (adjusted HR = 3.1; 95% CI 1.04-9.15, p<0.001)[14].

Liechty et al in a retrospective study done in rural Uganda on asymptomatic serum cryptococcal antigenemia and early mortality on 377 patients starting antiretroviral therapy reported that cryptococcal antigenaemia independently predicted death during the first 12 weeks of treatment [30]. The relative risk of death was 6.6%[95% confidence interval] baseline after controlling for CD4 count, viral load and other adverse prognostic markers**.** The population attributable risk for

mortality associated with a positive CRAG at baseline was 18% (CI 2–33%), similar to that associated with active tuberculosis (19%, CI 1–36%)[29].

Ina study by Meya et al 2010 in Uganda to determine the cost-effectiveness of serum cryptococcal antigen screening to prevent deaths among HIV-infected persons with a CD4+ cell count of 100 cells/ul who start HIV therapy in resource-limited settings, found that 26 patients (8.8%) out of 295 participants had cryptoccocal antigenemia. Amongst these patients 21 were promptly treated with fluconazole for 2-4 weeks, and of these patients 3 clinically developed C.M while in the remaining 5 CRAG positive patient not treated with fluconazole all died within 2 months of ART initiation. This demonstrated that ART alone was not sufficient to manage these patients with cryptoccocal antigenemia[19].

1.7 RISK FACTORS AND DETERMINANTS OF POSITIVITY

Severe immunosuppression assosiated with a low CD4 count of <100 cells/mm3 is assosiated with positive cryptococcal antigenaemia[27]. A study by Micol et al in Cambodia demostrated a mean CD4 count of 50 cells/mm3 was strongly assosiated with a positive serum CRAG. Other factors include: male gender, countryside residence, headache, and low BMI were all independently associated with positive serum cryptococcal antigen detection[28].

1.8 TREATMENT GUIDELINES FOR PREVENTION OF CRYPTOCOCCAL MENINGITIS

Guidelines released by the WHOrapid advice ondiagnosis, prevention and managementof cryptococcal disease in HIV-infected adults, adolescents and children 2011 recommend early ART initiation as the most important and cost-effective preventive strategy to reduce the incidence and high mortality associated with cryptococcalmeningitis in HIV-infected adults, adolescents and children. Ideally patients should initiate ART at a CD4 count of 350 cells/ mm^3, and definitely before a decline in the CD4 cell count to less than 200 cells/mm^3, consistent with WHO 2010 ART guidelines.

The use of routine serum or plasma CrAg screening in ART-naïve adults, followed by pre-emptive anti-fungal therapy if CrAg-positive, to reduce the development of cryptococcal disease, may be considered prior to ART initiation in:

a. patients with a CD4 count less than 100 cells/mm3, and

b. where this population also has a high prevalence of cryptococcal antigenaemia of >3%[21].

2.0 JUSTIFICATION

Cryptoccocal meningitis is the 2nd leading opportunistic infection in people living with HIV/AIDS (PLWHA) and is an AIDS defining illness with high morbidity and mortality. Routine screening and treatment of asymptomatic cryptoccocal disease prevents development of fulminant cryptococcal meningitis and therefore reduces mortality. Recent WHO guidelines advocate for routine screening for cryptococcal antigenaemia with targeted treatment in a section of those with low CD4 counts.

Treatment of cryptococcal meningitis requires the use of parenteral drugs in the intensive phase of treatment, and access to these drugs (5 flucytocine and amphotericin B) is limited in resource constrained settings. Fluconazole however, which is currently indicated in the treatement of asymptomatic cryptocogenemia, is widely available in these settings.

The cost of screening and treatment of cryptoccocal antigenemia may therefore be less than the treatment of cryptoccocal meningitis, making it a more cost effective intervention.

There is a paucity of data on the prevelance and standardization of management of cryptococcal antigenemia locally.

Results of this study will form a useful part in developing a data base of such patients who may benefit from screening and treatment while still asymptomatic, as advocated in recently published guidelines. These results may also contribute to the development of a policy for the implementation of pre–emptive antifungal therapy for cryptoccocal disease in HIV-infected cohorts in Nairobi.

3.0 RESEARCH QUESTION

What is the prevalence of cryptococcal antigenemia in severe immunosuppresed HAART naïve HIV-infected adults?

3.1 BROAD OBJECTIVE

To determine the prevalence of cryptoccocal antigenemia and assosiated factors in HIV-infected adult in-patients, HAART naive with low CD4 counts at the Kenyatta National Hospital and Mbagathi hospital.

3.2 PRIMARY OBJECTIVE

1. To determine the prevalence of serum cryptococcal antigenemia in HIV-infected adults, HAART naïve, with a CD4 count of <100 cell/µL admitted to the medical wards at the KNH and Mbagathi hospitals.

3.3 SECONDARY OBJECTIVES

1. To determine the association between the demographic factors and cryptoccocal antigenemia.

2. To determine assosiation between clinical characteristics (symptomatology),WHO staging and laboratory characteristics(CD4 count) with cryptococcal antigenemia.

4.0 METHODOLOGY

4.1 STUDY DESIGN

Cross sectional descriptive survey.

4.2 STUDY SITE

Kenyatta National Hospital and Mbagathi District Hospital medical wards.

4.3 STUDY POPULATION

Adults 18 years and above,HIV positive, admitted in the medical wards, HAARTnaïve, and with a CD4 count of <100 cells/mm³.

4.4 SAMPLE SIZE CALCULATION AND SIZE

For this study the sample size required was calculated according to the following formula.

$$n = \frac{(Z_{1-\alpha/2})^2 \left(p(1-p)\right)}{d^2}$$

n= Sample size

$Z_{1-\alpha/2}$= Statistic for the level of confidence of 95%, 1.96

p= Estimated prevalence of cryptococcal antigenemia of15% based on regional data and study done by Wajanga in Tanzania[26]

d= Precision, 0.05

The required sample size was196 patients.

4.5 INCLUSION CRITERIA

Patients who gave written informed consent.

HIV positive patients with a CD4 count of<100 cell/µL.

HAART naïve adult(18 years and above) patients.

4.6 EXCLUSION CRITERIA

Those who declined to give consent.

Prior history of treatment for cryptococcal disease in the last 6 months.

Current or prior use of high dose fluconazole in the last 6 months.

4.7 STUDY VARIABLES

Dependent variable:

- Serum cryptoccocal antigen

Independent variable:

1.Demographic data

- Age
- Gender

2. Clinical data

- Clinical characteristics ;Symptoms of meningitis (headache, fever, neck stiffness, altered sensorium, photophobia and projectile vomiting)
- WHO staging
- CD4 counts

4.8 STUDY FEASIBILITY

The study washospital based conducted at the KNH and Mbagathi medical wards, where an average of 80 patients per month were seen who met the inclusion criteria. A total of 7 medical wards were in KNH, each ward admited an average of 2 patients every week who met the inclusion criteria It therefore took 14 weeks to reach the desired sample size of 196 patients.

4.9 PRETESTING OF THE STUDY PROFORMA

The questionnaire was pretested on a randomly selected sample of about 20 HAART naïve HIV-infected adult patients at the medical wards in Kenyatta National and Mbagathi District Hospital two weeks before the actual study. The aim of the pretesting was to have clarity in the questions to be put forth to the respondents. The pretest alsoaimed to assess the flow, order, skip patterns, timing, and overall respondents' well-being. There after the questions and questionnaire was drawn into its final form. Participants in the pretesting of the questionnaire were not included in the final data analysis

4.10 METHODS

The study protocol was approved by the KNH/UON Ethical and Research Committee. HIV-infected HAART naïve adult patients with a CD4 count of <100cell/µL. were recruited consecutively from a population of patients admitted to the medical wards at Kenyatta and Mbagathi Hospital. HIV-infected patients were identified following a positive serology test for HIV-1 and 2 using the standard testing technique (Unigold and Bioline and confirmed by ELISA).

In the medical wards, the principle investigator or her assistant went to the post admission ward. All the files of the new admissions were reviewed by the PI or her assistant. Patients found to be HIV positive were fast tracked to have the CD4 count done. Those found to have a CD4 count of <100 cell/mm^3, HAART naïve and had no prior history of either cryptococcal disease and use of high dose fluconazole underwent counseling and explanation of the study procedure. Signed

informed consent was thenobtained. The principal investigator obtained consent from the primary doctor or the next of kin for patients with altered level of consciousness.

A study proforma was then be administered by the assistant or the principal investigator to all enrolled participants. The questionnaire was administered in either English or Kiswahili. Patients thenunderwent a thorough physical examination to look for symptoms and signs of meningitis and to ascertain WHO clinical staging.

A 2mL sample of blood from each identified participant was collected aseptically, dispensed into a plain vacutainer® tube labeled appropriately. The CRAG test was done by a lateral flow assay kit as described in appendix 1 and results recorded on the study proforma.

Patients found to have symptoms and signs suggestive of meningitis underwent fundoscopy done by the PI using the described procedure in appendix 4a.A lumbar puncture was done by the PI in a standard fashion as described in appendix 4b. Cerebral spinal fluid CRAG was done at the immunology laboratoryand results communicated to the primary doctor and put in the patient file.

Patients with cryptococcal antigenemia were referred to the primary doctor for appropriate management withoral fluconazole and initiation of HAART after at least 2 weeks of fluconazole (a standard treatment protocol with current guidelines was attached to the patient's file and communicated to the primary doctor).Patients with positive CSF antigenemia were treated as cryptococcal meningitis using the standard treatment protocol

4.11 LABORATORY PROCEDURES

Serology tests for the serum CRAG were done using the lateral flow assay kit; an ELISA based method, at the University of Nairobi Immunology laboratory. This was done by a qualified laboratory technician. Patients were referred to as serum CRAG positive if they tested positive by this method.

CD4 counts were done by the CyFlow@counter machine in the laboratory.

4.12 QUALITY ASSUARANCE

The KNH and Mbagathi hospital laboratories have a well stipulated quality assurance protocol which was adhered to. In addition a qualified laboratory technician was trained on how to administer and interpret the test. Quality assurance on serum CRAG serology was as per manufacturers' recommendation.

4.13 DATA ANALYSIS AND MANAGEMENT

Data was collected using a standardized study proforma. The collected data was entered into excel spread sheets and analysis was done using the statistical package SPSS version 17.0. The data was cleaned for errors and inconsistent (conflicting) answers, missing entries and duplicate entries to ensure high quality data.

Descriptive statistics on continuous data (age and CD4 counts) was presented as means, median, standard deviations, and interquartile ranges. Categorical data (CRAG sero-positivity, gender, symptomatology and WHO staging) was analyzed as percentages and frequencies. Chi-square test was used to assess association between CRAG positivity and categorical variables. Students' T-Test was used to assess the association between CRAG positivity and continuous variables. An odds ratio (OR)was used to measure the magnitude of association. Level of significance was 0.05.

5.0 RESULTS

In July 2012 to October 2012,196 HAART naïve in-patients were recruited into the study as show in the flow diagram below.

Figure 1: Flow Chart on Screening and Recruitment of Patients

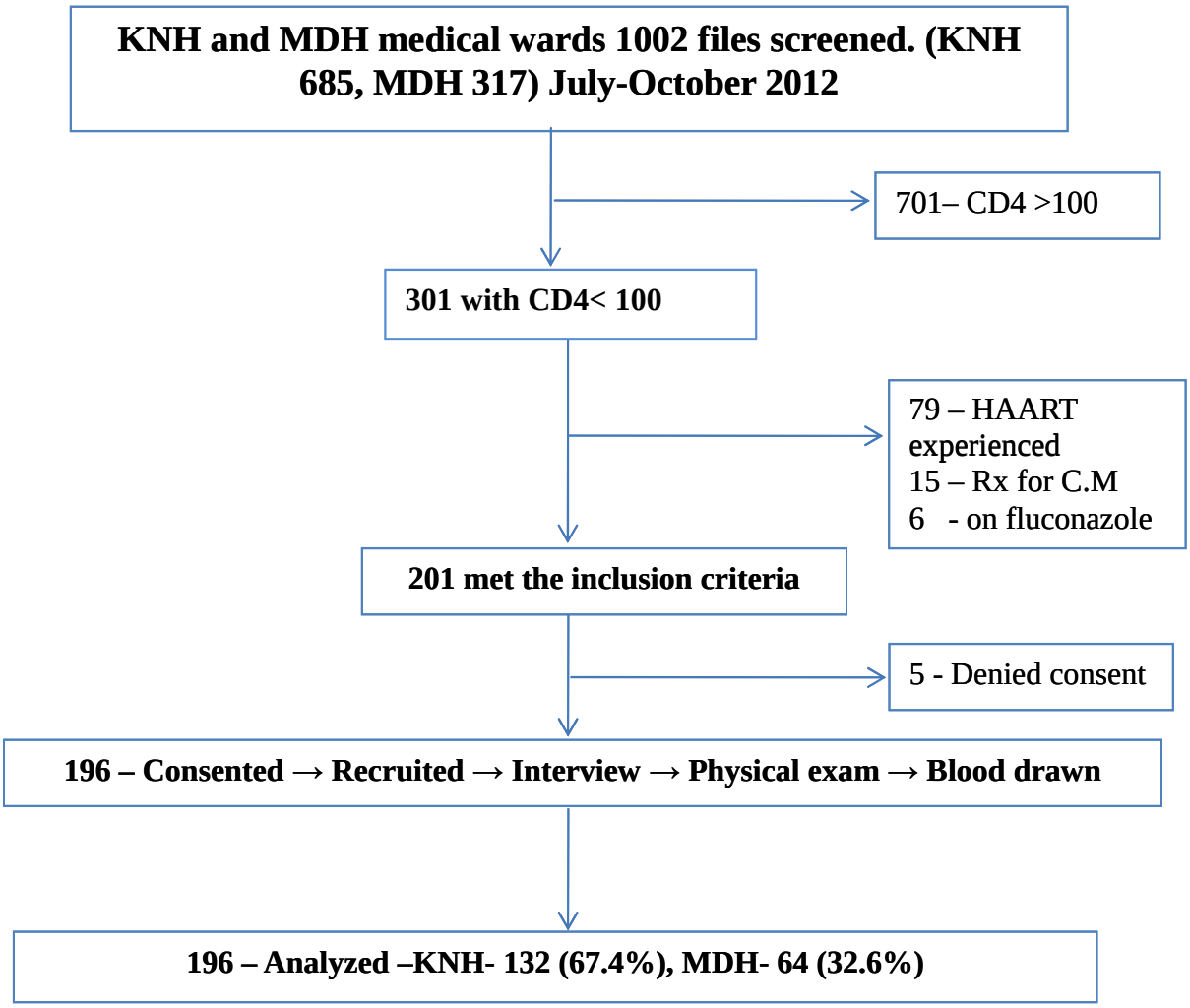
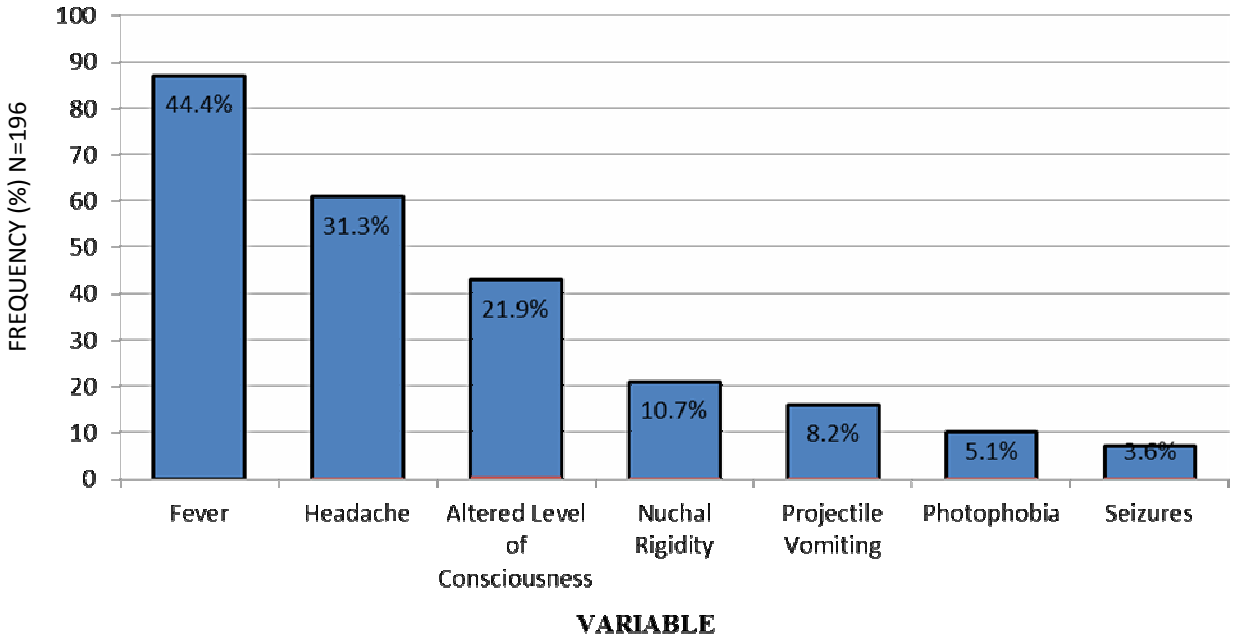


Table 1: Baseline Demographic and Clinical Characteristics

VARIABLE	FREQUENCY (%) N=196
AGE in years	
• Mean (SD)	37.4 (9.1)
• Median (IQR)	38.0 (32.0-43.5)
• Min-Max	19-67
GENDER	
• Male	82 (41.8%)
• Female	114 (58.4 %)
RESIDENCE	
• Urban	165 (84.5%)
• Rural	31 (15.8 %)
OCCUPATION	
• Formal	60 (30.6%)
• Casual	136 (64.4%)
WHO STAGING	
• Stage 1	29 (14.8%)
• Stage 2	12 (6.1%)
• Stage 3	85 (43.3%)
• Stage 4	70(35.7%)
CD4 COUNT (cell/µl)	
• Mean (SD)	42.0 (32.6)
• Median (IQR)	34.0 (12.0-76.0)
• Min-Max	0-100

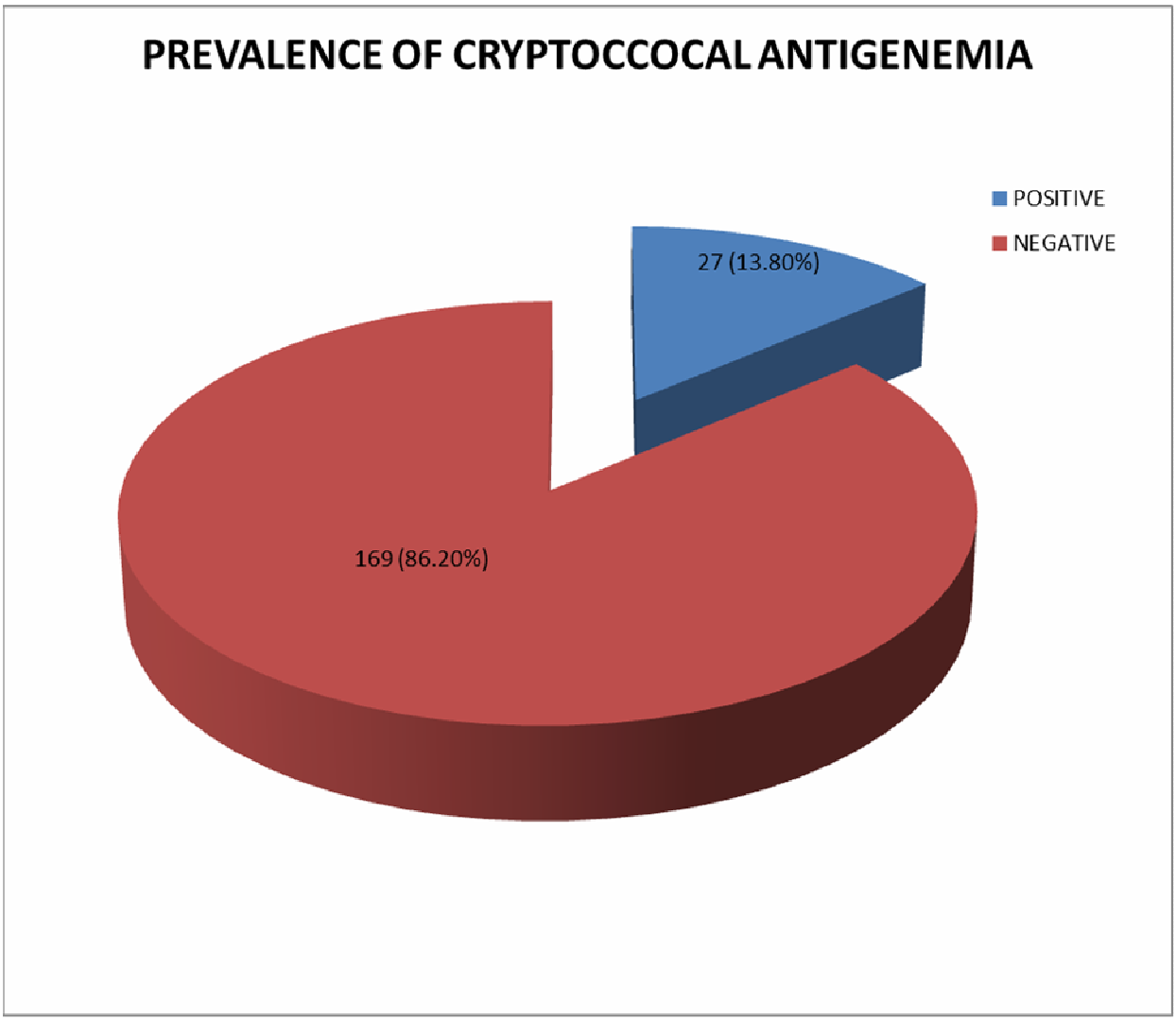
The mean age of the study population was 37 years with a median age of 38 yearswith an age range of 19-67 years. Female patients constituted the majority at 58.2%.Majority of the patients were from Nairobi and its environs and were involved in a casual occupation. Most patients were in stage 3 and 4 WHO clinical staging at 43.3% and 35.7% respectively. A median CD4 count of 34 cell/µl was obtained with a range of 0 to 100.

Figure 2: Clinical Characteristics



As this was an in-patient study all the patients had at least one sign or symptom of meningeal irritation.Majority of the patients had fever,headache,altered level of conciousness and neck stiffness.

Figure 3: Prevalence of cryptococcal-antigenemia



The prevalence of cryptococcal antigenemia was 13.8% with a 95% CI of 9.2-18.9.

Out of the 27 patients with cryptococcal antigenemia,24 patients underwent a lumbar puncture.The remaining 3 patients died before a lumbar puncture could be performed. 19 patients (80%) had positive CSF CRAG. 5(20%) patients had negative CSF CRAG.

Table 2: Opportunistic infections in the study population

WHO CLINICAL STAGING	OPPORTUNISTIC ILLNESS	N=196
Stage 1	Asymptomatic	5
	Generalised Lymphadenopathy	24
Stage 2	Herpes zoster	6
	Papular pruritic erruptions	4
	Seborrhoeic dermatitis	2
Stage 3	Chronic diarrhea	10
	Oral candidiasis	10
	Pulmonary tuberculosis	51
	Community acquired pneumonia	7
	Bacterial meningitis	6
	Septicaemia	1
Stage 4	Extra pulmonary TB	32
	PCP	10
	Esophageal candidiasis	6
	Kaposi sarcoma	5
	CNS toxoplasmosis	4
	PML	2
	CMV retinitis	1
	B- cell non-Hodgkin Lymphoma	3
	HIV associated nephropathy	7

Majority of the patients were in WHO stage 3.The commonest opportunistic infection was tuberculosis 84 (42.8%).

Table 3: Demographic factors associated with cryptococcal antigenemia

Variable	Serum CRAG		OR (95% CI)	P value
	Positive	Negative		
Age, mean (SD)	39.7 (9.6)	37.5 (9.0)	-	0.271
Gender				
Male	15 (55.6)	67 (39.6)	1.9 (0.8-4.3)	0.120
Female	12 (44.4)	102 (60.4)	1.0	
Residence				
Urban	21 (77.8)	144 (85.2)	0.6 (0.2-1.7)	0.392
Rural	6 (22.2)	25 (14.8)	1.0	

Majority of the patients with cryptococcal antigenemia were male (55.6%) and resided in Nairobi and its environs.These demographic factors however,did not achieve statistical significant association.

Table 4: Clinical characteristic(symptomatology),WHO staging and laboratory characteristic (CD4 count) associated with cryptoccocal antigenemia

Variable	Serum CRAG		OR (95% CI)	P value
	Positive	Negative		
Headache				
Yes	11 (40%)	50(29.6)	1.6 (0.7-3.8)	0.245
No	16(59.3%)	119 (70.4)	1.0	
Fever				
Yes	16 (59.3)	71 (42.0)	2.0 (0.9-4.6)	0.094
No	11 (40.7)	98 (58.0)	1.0	
Neck Stiffness				
Yes	7 (25.7)	14 (8.3)	3.9	
No	20 (74.1)	155(91.7)	1.0(1.4-10.7)	0.013
Altered level of consciousness				
Yes	10 (37.0)	33 (19.9)	2.4 (1.0-5.7)	0.047
No	17 (63.0)	133 (80.1)	1.0	
WHO clinical staging				
Stage 1 and 2	6 (22.2)	35 (20.7)	0.9 (0.3-2.4)	
Stage 3 and 4	21 (77.8)	134 (79.3)	1.0	0.858
CD4 count (cells/µl)				
≤ 50	20 (74.1)	101 (60.8)	1.8 (0.7-4.6)	0.187
>50	7 (25.9)	65 (39.2)	1.0	

Majority of the patients with cryptococcal antigenemia were in WHO stage 3 and 4 and had a CD4 count of ≤ 50 cell/µl (74 %).Majority of these clinical characteristics did not achieve statistical significance.In the bivariate analysis of cryptococcal antigenemia and clinical characteristics, patients with neck stiffness were almost 4 times more likely to have cryptococcal antigenemia with a significant p-value of 0.01.

6.0 DISCUSSION, CONCLUSION & RECOMMENDATION

6.1 DISCUSSION

We set out to establish the prevalence of cryptoccocal antigenemia and its assosiated factors in Kenyatta National and Mbagathi Hospitals in view of the WHO rapid advice guidelines 2012 that reccomended screening and pre-emptive treatement of patients in areas endemic with a prevalence of > 3%.The prevalence in our set up largely remains unknown.

The study population comprised of fairly young people,with a mean age of only 37 years.This is not suprising as local and international studies done regarding HIV and its complications have demonstrated the same. Tsuma et al for his Mmed dissertation undertook a cross sectional study in Kenyatta National Hospital to determine the Prevalence of kaposis sarcoma in HAART naïve HIV patients found the mean age to be 37 years.Nationally according to the Kenya Aids Demographic Survey (KAIS)2007 demonstrated a higher proportion of kenyans aged 30-36 years compared to other age groups were infected with HIV.This age group contributes significantly to the economy of the country hence more preventive policies are needed, tailored to target this age group.

Table 1shows females were the majority in a ratio of 1:1.4 mimicking previous experiences in Kenya where females get HIV and its complications at a relatively young age compared to their male counterparts. KAIS 2007 estimates that majority of HIV infected adults are women in a ratio of 1:1.6,hence more emphasis is needed on targeting more resources in the prevention and treatement of this vulnerable gender. In addition the study population resided in Nairobi and its environs and majority were in casual employment.

In this study we targeted patients with a CD4 count of <100 cells/mm^3 as cryptococcal infection is an AIDS defining illness common at this CD4 count.Majority of the patients had a CD4 count of < 50 cell/mm^3.These patients were newly diagnosed and HAART naïve presenting with clinically and immunologically advanced disease despite country-wide voluntary testing initiatives.

As this was an in-patient setting, all the patients had one or more symptom or sign to suggestive of meningeal irritation however,the patients had a wide variety of opportunistic infections that could present with the same symptomatology .The most common symptom alone or in combination were fever, headache and neck stiffness at 44.4%,31,1% and 10.7% respectively.This was similar to a cross sectional study done by Micol et al in Cambodia where he studied determinants of positivity and clinical utility of cryptoccocal antigenemia in HIV patients and found fever,headache and neck stiffness to be common at 80%,57.2% and 18.6% respectively[28].In our study,80% of patients with cryptococcal antigenemia had a positive CSF CRAG and were treated as cryptococcal meningitis .The remaining 20% of the patients had a negative CSF CRAG .These patients were treated with pre-emptive antifungal treatment.This finding is similar to a study done by Oyella et al in Uganda .Therefore in view of the WHO rapid advice guidelines that recommend treatement in asymptomatic patients with oral fluconazole in areas with a prevalence cryptococcal antigenemia of >3 %,a larger study done in an out-patient setting would be able to establish the prevalence of patients who are asymptomatic.

We found a cryptoccocal antigenemia seroprevalence of 13.8 % in this urban setting in HIV infected in-patients with severe immunosuppression.This was consistent globally and also with several studies done in the developing world.A cross sectional study done by Wanjaga et al in Tanzania demonstrated a prevalence of 15%[26], Oyella et al in Uganda demonsrated a prevalence of 19%[27] and Micol et al in Cambodia showed a prevalence of 19%[28].In contrast studies done in the developed countries have reported a low seroprevalence of cryptoccocal antigenemia. HIV patients in the developed world are diagnosed and started on HAART early and rarely present with advanced disease.Locally the scenario is different whereby patients present with low CD4 counts and advanced WHO clinical staging in particular tuberculosis which alters cell mediated immunity that is key in the pathogenesis of cryptoccocal disease .In addition,there is geographic variation in the distributionof cryptococcal neoformans,in USA and Canada *cryptoccocal neoformans var gatii* is mainly found in areas with eucalyptus trees while in developing countries *cryptoccocal neoformans var neoformans* is found in avian feceas and spread via droplet infection.*Cryptoccocal neformans var neoformans* serotype A is the causative organism in cryptoccocal menigitis in patients with AIDS.

Cryptococcal antigenemia is an independent predictor of mortality[14] therefore the high prevalence in our study is worrying and may require the need for screening programs to diagnose cryptococcal infection among in-patients with severe immunosuppression prior to the initiation of ART in tune with the WHO rapid advice guidelines 2012.In patients with one or more meningeal symptom a serum and CSF CRAG should be undertaken in order to distinguish cryptococcal meningitis and cryptoccocal antigenemia as each has a different management protocol. Several cohorts in sub-saharan Africa have reported high early mortality and immune reconstitution inflamatory syndrome (IRIS) after ART initiation[23 24].In one study a high mortality of 14% was reported.Tuberculosis and cryptoccocal disease were the leading opportunistic illnesses that caused IRIS and high early mortality [22].Therefore screening may offer an oppprtunity to reduce unmasking form of IRIS and the mortality assosiated.

Although this study was not powered to determine the secondary objectives, neck stiffness was found to be assosiated with cryptoccocal antigenemia.This is similar to studies done in Uganda and Tanzania[26] [27].Majority of these patients had cryptoccocal meningitis.Severe immunosuppression predisposed these patients to developing this stage 4 AIDS event.

In Table 4, study participants with a CD4 count of <50 cell/mm³ were more likely to have cryptoccocal antigenemia (74.1%)compared with (25.9%) of patients with CD4 count of ≥50 cell/mm³(OR 1.8 with 95% CI).This finding is similar to that reported in Cambodia[28].This is attributable to dysfunctional immune systems that predispose patients with CD4 count of <50 cell/mm³ to cryptoccocal infection.Therefore in constainted resource settings screening for cryptoccocal antigenemia may be targetted these patients with CD4 of <50 cell/mm³.

6.2 CONCLUSION

Cryptococcal antigenemia is common in HIV positive patients with advanced immunosuppression.

6.3 LIMITATIONS

This was an in-patient study largely carried out in Kenyatta National Hospital a refferal facility and the results obtained may not be applicable to out-patient settings.

The study population was recruited from a limited geographical area and therefore not generalisable to the other regions in Kenya.

6.4RECOMMENDATIONS

Routine screening may be considered in HIV positive,HAART naïve in-patients with CD4 counts of <50 cell/mm^3

Large out-patient studies need to be carried out to determine the prevalence of asymptomatic cryptoccocal antigenemia

7.0REFERENCES

1. UNAIDS/WHO AIDS epidemic update 2007

2. French N, Gray K, Watera C. Cryptococcal infection in a cohort of HIV-1-infected Ugandan adults. *AIDS.*2002; 16(7):1031-8.

3. Chariyalertsak S,Sirisanthana T. Clinical presentation and risk behaviors of patient with AIDS in Thailand. *Clinical infectious disease* .2001; 32(6); 955-962.

4. Gordon SB, Walsh L, Chaponda M. Bacterial meningitis in Malawian adults: pneumococcal disease is common, severe, and seasonal. *Clinical infectious disease.* 2000; 31(1):53-7.

5. Békondi C, Bernede C, Passone N. Primary and opportunistic pathogens associated with meningitis in adults in Bangui, Central African Republic, in relation to human immunodeficiency virus serostatus. *International journal of infectious diseases*. 2006; 10(5):387-95.

6. Hakim JG, Gangaidzo IT, Heyderman RS. Impact of HIV infection on meningitis in Harare, Zimbabwe: a prospective study of 406 predominantly adult patients. *AIDS.*2000; 14(10):1401-7.

7. J. O Jowi, P. M Mativo S. Clinical and laboratory characteristics of hospitalized patients with neurological manifestations of HIV at the Nairobi hospital. *EAMJ*. 2007; 84(2):66-76.

8. Bicanic T, Wood R, Meintjes G. High-dose amphotericin B with flucytosine for the treatment of Cryptococcal meningitis in HIV-infected patients. *Clinical infectious diseases*.2008; 47(1):123-30.

9. Lortholary O, Poizat G, Zeller V. Long-term outcome of AIDS-associated cryptococcosis in the era of combination antiretroviral therapy. *AIDS.*2006; 20(17):2183-91.

10. Mirza S, Phelan M, Rimland D, et al. The changing epidemiology of cryptococcosis: an update from population-based active surveillance in 2 large metropolitan areas, 1992–2000. *Clinical infectious diseases*. 2003; 36(6):78.

11. Longley N, Muzoora C, Taseera K, *et al*. Dose response effect of high-dose fluconazole for HIV-associated Cryptococcal meningitis in southwestern Uganda. *Clinical infectious diseases*. 2008; 47(12):1556-61.

12. Nussbaum JC, Jackson A, Namarika D, *et al*. Combination flucytosine and high-dose fluconazole compared with fluconazole monotherapy for the treatment of Cryptococcal meningitis: a randomized trial in Malawi. *Clinical infectious disease*. 2010; 50(3):338-44.

13. French N, Grey Christine W, Sessik N. Cryptococcal Infection in a Cohort of HIV-1 Infected Ugandan Adults. *AIDS* 2002, 16:1031-1038.

14. Jarvis JN, Lawn SD, Vogt M, *et al*.UKPMC Funders Group Screening for Cryptococcal Antigenemia in Patients Accessing an Antiretroviral Treatment Program in South Africa. *Clinical Infectious Diseases*. 2009; 48(7):856-862.

15. Bicanic T, Muzoora C, Brouwer AE. Independent association between rate of clearance of infection and clinical outcome of HIV-associated Cryptococcal meningitis: analysis of a combined cohort of 262 patients. *Clinical infectious diseases*. 2009; 49(5):702-9.

16. Park BJ, Wannemuehler KA, Marston BJ. Estimation of the current global burden of Cryptococcal meningitis among persons living with HIV/AIDS.*AIDS*. 2009; 23(4):525-30.

17. Twaspejulahaj T, Hajis M, Schwitswarek P. Demonstration of encapsulated yeast in India ink preparation: *Journal of Microbiology* 2003; 26(6):54-67.

18. Feldmesser M, Harris C, Reichberg S, *et al*. Serum Cryptococcal antigen in patients with AIDS. *Clinical infectious disease*. 1996; 23(4):827-30.

19. Meya DB, Manabe YC, Castelnuovo B. Cost-effectiveness of serum Cryptococcal antigen screening to prevent deaths among HIV-infected persons with a CD4+ cell count

< or = 100 cells/microL who start HIV therapy in resource-limited settings. *Clinical infectious diseases*. 2010; 51(4):448-55.

20. Bicanic T, Meintjes G, Wood R. 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 disease.*2010; 51(4):448-55.

21. WHO Rapid advice Diagnosis, Prevention and Management of Cryptococcal Disease in HIV-infected adults, adolescents and children. 2011 ;(December).

22. Hirsch HH, Kaufmann G, Sendi P, Battegay M. Immune reconstitution in HIV-infected patients. *Clinical infectious diseases*. 2004; 38(8):1159-66.

23. Lortholary O, Fontanet A, Mémain N. Incidence and risk factors of immune reconstitution inflammatory syndrome complicating HIV-associated cryptococcosis in France. *AIDS.*2005; 19(10):1043-9.

24. Shelburne SA, Darcourt J, White AC. The Role of Immune Reconstitution Inflammatory Syndrome in AIDS- Related Cryptococcus neoformans Disease in the Era of Highly Active Antiretroviral Therapy. *AIDS*. 2005; 7703:1049-1052.

25. Odhiambo N, Kendi C, Meyer AC, Penner J. FACES. Feasibility of Decentralized Scale Up of Routine Cryptococcal Screening and Treatment for patients with CD4 count of <100 cell/mm³ in Kenya.

26. Wajanga BMK, Kaluga Downs JA. Universal screening of Tanzanian HIV Infected Adults in patients with serum Cryptococcal Antigen to Improve Diagnosis and Reduce Mortality: an observational study. *Journal of the International AIDS Society*,2011;14(48)1-7

27. Oyella J, Meya D, Bajunirwe F, *et al,* Prevalence and factors associated with cryptococcal antigenemia among severely immunocompromised HIV infected adults in Uganda ;a cross sectional study. *Journal of the International AIDS Society*2012 15;15 1-7

28. Micol R, Lortholary O, Sar B. Prevalence, determinants of positivity, and clinical utility of Cryptococcal antigenemia in Cambodian HIV-infected patients. *Journal of acquired immune deficiency syndromes (1999*). 2007; 45(5):555-9.

29. Liechty C, Solberg P, Were W. Asymptomatic serum Cryptococcal antigenemia and early mortality during antiretroviral therapy in rural Uganda. *Tropical medicine & international health*. 2007; 12(8):929-35.

30. Lawn SD, Harries AD, Anglaret X, et al. Early mortality among adults accessing antiretroviral treatment programs in sub-Saharan Africa. *AIDS*. 2008; 22(15):1897-908.

31. Chariyalertsak S, Sirisanthana T. Clinical Presentation and Risk Behaviors of Patients with Acquired Immunodeficiency Syndrome in Thailand, 1994 – 1998: Regional Variation and Temporal Trends. *Clinical infectious*. 2001; 32(6):955-962.

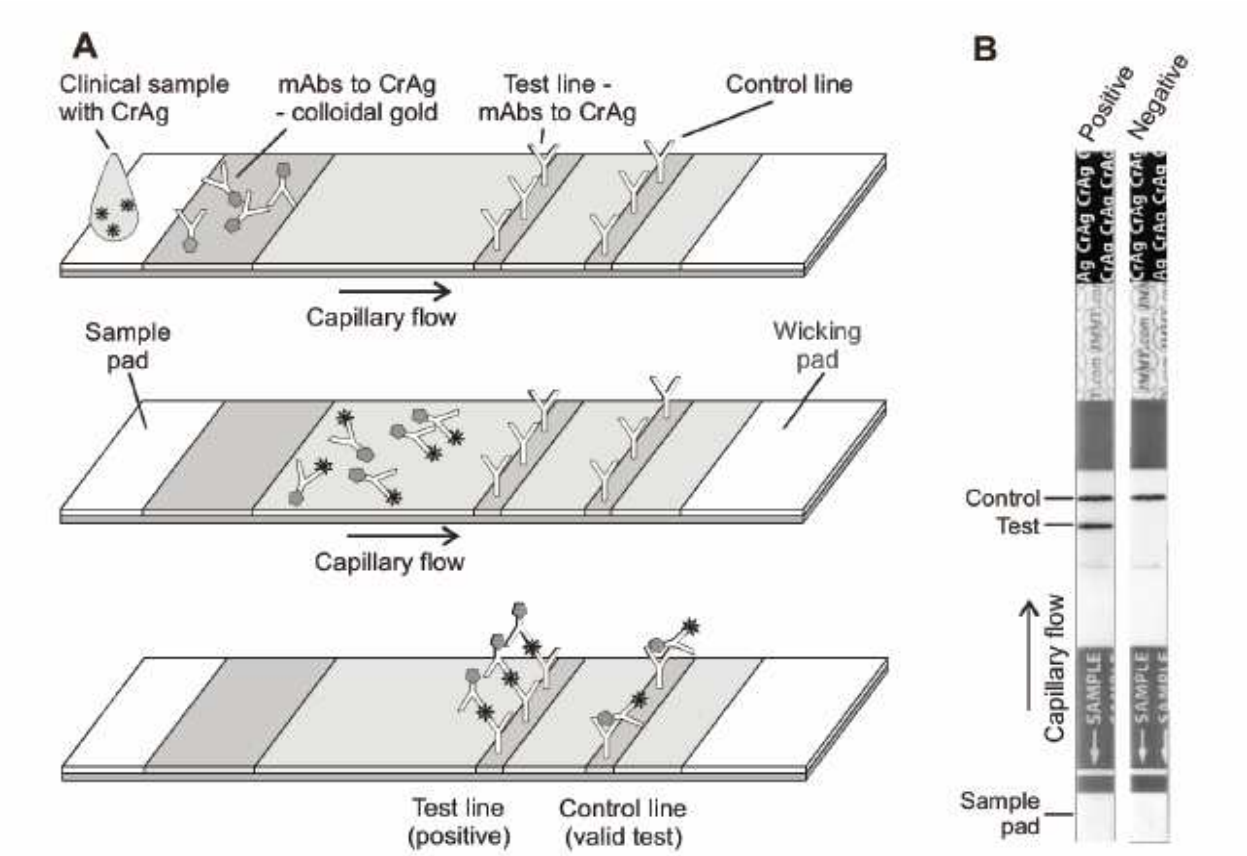
APPENDIX 1: CrAgLateral FLOW ASSAY

The CrAg LFA is manufactured by Immuno Mycologic Inc USA.The WHO noted that the LFA had several key advantages. These include lower cost, rapid turnaround time, minimal training required, and can be performed with minimal laboratory infrastructure. Most importantly for the developing world, the CrAg LFA is the only test onthe market that meets allof the WHO's ASSURED criteria, meaning it is an assay that can be easily implemented in developing world labs.

Principle of the Procedure

TheCrAgLateralFlowAssayisadipstick sandwich immune chromatographic assay, whichdetects cryptococcal antigenin serum. For thequalitativeprocedure, specimensarediluted1:2in1xspecimen diluents andanalyzed. Forthe semi-quantitativeprocedure, specimens arediluted1:5in1xspecimen diluents followedby1:2serialdilutions. Alldilutionsarethen analyzedasinthequalitativeprocedure. Aspecimen(s)isplaced into anappropriatereservoir(s), suchasatesttube(s) oramicro titer plate(s), and thelateralflowdevice Isthenplacedintothereservoir(s), allowingthespecimen(s)tocomeintocontactwiththetest membrane(s).Thetestusesspecimenwickingtocapture gold conjugated, anti-cryptococcal monoclonalantibodiesandgold conjugatedcontrolantibodiesthataredepositedontoamembrane. Ifcryptococcalantigenispresentinthespecimen, itbindstothegold–conjugated,anti-cryptococcalantibodies. Thegoldlabeledantibodyantigencomplexwillcontinuetowick upthemembranewhereItwill interact withthetest line (immobilizedanti-cryptococcal monoclonal antibodies).Ifthespecimencontainscryptococcalantigen, asandwichiscreatedwiththegold-labeledantibodiesandtheimmobilizedantibodies, causingavisiblelinetodevelopatthetest linesite. Ifproperflowoccursandthe reagentarereactiveat thetimeofuse, thewickingofanyspecimen, positiveornegative, willcausethegold-conjugatedcontrolgoatIgGantibodytomovetothecontrolline(immobilizedbovineanti-goatIgGantibody).Theimmobilizedanti-goatantibodywillbindtothegold-conjugatedgoatIgGcontrolantibodyandwillcauseavisiblelinetodevelop.

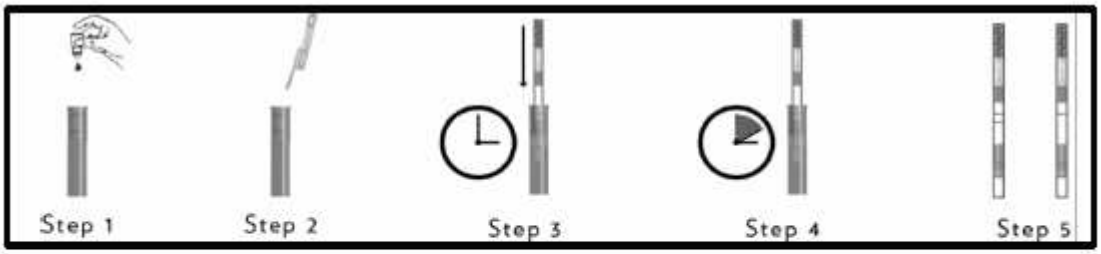
A positive test result will create two lines, while a negative test result will create one line (Figure B).If the controlline fails to develop, the test is not valid.



(A) Schematic showing operation of the CrAg lateral flow assay (LFA). LFA is constructed from monoclonal antibodies (mAbs) specific for CrAg. (B) Positive and negative samples on the CrAg LFA. Presence of two lines is a positive test, and one line is a negative test.

CrAg Lateral Flow Assay: Method

One major advantage of CrAg LFA over other methods currently available cryptococcal antigen detection assays such as LA or EIA is its ease of use. After five easy steps, results are obtained in 10 minutes. The LFA does not require specimen pre-treatment .It does not require equipment and is easily scaled up to handle 30 samples or more. The assay is also semi quantitative allowing for titre determination.



Five easy steps are all that is required to perform the CrAg LFA. **Step 1**: Add one drop of specimen to a tube. **Step 2**: Add of 40 ul of patient specimen to the tube. **Step 3**: The CrAg LFA strip is inserted into the tube. **Step 4**: Incubate for 10 minutes. **Step 5**: Interpret results.

APPENDIX 2: WHO CLINICAL STAGING OF HIV/AIDS FOR HIV INFECTED ADULTS AND ADOLESCENTS

PRIMARY HIV INFECTION

Asymptomatic

Acute retroviral syndrome

STAGE 1

Asymptomatic

Persistent generalized lymphadenopathy

STAGE 2

Moderate unexplained weight loss (<10% of presumed or measured body weight)

Recurrent respiratory infections (sinusitis, tonsillitis, otitis media, and pharyngitis)

Herpes zoster

Angular cheilitis

Recurrent oral ulceration

Papular pruritic eruptions

Seborrheic dermatitis

Fungal nail infections

STAGE 3

Unexplained severe weight loss (>10% of presumed or measured body weight)

Unexplained chronic diarrhea for >1 month

Unexplained persistent fever for >1 month (>37.6ºC, intermittent or constant)

Persistent oral candidiasis (thrush)

Oral hairy leukoplakia

Pulmonary tuberculosis (current)

Severe presumed bacterial infections (e.g., pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteremia)

Acute necrotizing ulcerative stomatitis, gingivitis, or periodontitis

Unexplained anemia (hemoglobin <8 g/dL)

Neutropenia (neutrophils $<0.5x10^9$/L)

Chronic thrombocytopenia (platelets $<50x10^9$/L)

STAGE 4

HIV wasting syndrome

Pneumocystis pneumonia

Recurrent severe bacterial pneumonia

Chronic herpes simplex infection (orolabial, genital, or anorectal site for >1 month or visceral herpes at any site)

Esophageal candidiasis (or candidiasis of trachea, bronchi, or lungs)

Extra pulmonary tuberculosis

Kaposi sarcoma

Cytomegalovirus infection (retinitis or infection of other organs)

Central nervous system toxoplasmosis

HIV encephalopathy

Cryptococcosis, extra pulmonary (including meningitis)

Disseminated nontuberculosis mycobacteria infection

Progressive multifocal leukoencephalopathy

Candida of the trachea, bronchi, or lungs

Chronic cryptosporidiosis (with diarrhea)

Chronic isosporiasis

Disseminated mycosis (e.g., histoplasmosis, coccidioidomycosis, penicilliosis)

Recurrent nontyphoidal *Salmonella* bacteremia

Lymphoma (cerebral or B-cell non-Hodgkin)

Invasive cervical carcinoma

Atypical disseminated leishmaniasis

Symptomatic HIV-associated nephropathy

Symptomatic HIV-associated cardiomyopathy

Reactivation of American trypanosomiasis (meningoencephalitis or myocarditis)

APPENDIX 4: PROCEDURE FOR FUNDOSCOPY

4a: Procedure for fundoscopy

1. Patient will be in a dark examining room (prevents constriction of pupils).
2. The patient will be placed in a comfortable position before starting the exam.
3. The patient will be given a specific object on the wall on which to fixate (prevents constriction of pupils from accommodation).
4. The ophthalmoscope will be turned on to a low-moderate light intensity, using the smallest aperture to look into theundilated eye, and the largest aperture to observe a dilated eye.
5. The hand that is not holding the ophthalmoscope will be either on the patient's head or shoulder to help you judge your distance.
6. The right eye and right hand will look into the patient's right eye (and the left eye/left hand will be used for the patient's left eye).
7. Looking through the ophthalmoscope into the patient's eye from a distance, the red reflex will be found.
8. Following the red reflex into the eye at a small angle towards the patient's nose; the examiner will focus on the optic disc and follow the superonasalarcade, followed by the inferonasal arcade, the superotemporal arcade and the inferotemporal arcade respectively.
9. Focus on the macula (temporal to the optic disc) will be done.
10. The examiner will make a note on the findings. A finding of papilledema will contraindicate a lumbar puncture.

Appendix 4b: Procedure for lumbar puncture.

1. Informed consent will be obtained from the patient or next of kin.
2. A CT scan of the head or a fundoscopic exam to check for papilledema will be obtained to rule out increased intracranial pressure before proceeding.
3. Patient will be placed in a sitting position on the edge of the bed (much like the position for a spinal or epidural) or in a lateral recumbent position (lying onthe side with knees tucked to chest and chin to chest)
4. The L3-L4 space will be located by palpating for the iliac crestand moving you're fingers medially from the crests to the spine.
5. The entry site will be marked with the thumbnail or a marker.
6. The spinal tray will be opened and prepared in a sterile manner.
7. Using the skin swabs and sterile antiseptic solution to clean the skin at thechoseninterspace will be cleaned along with the space below (in case you need to move to the lower space after a failed attempt). Clean the L3-L4 space in a circular fashion starting at the center and moving outward
8. The sterile drape will be placed on the patient.
9. Using a 25-gauge needle and the 3-cc syringe to the 1% lidocaine will be administered intradermally, creating a skin wheal.
10. The plastic numbered test tubes will be opened andplaced upright in the preformed circular slots in the tray whilewaiting for the lidocaine to take effect.
11. The spinal needle (20- or 22-gauge) will be placed through the skin wheal between the L3 and L4 spinous processes at a slightly cephalad angle toward the umbilicus.
12. The needle will be advanced slowly but smoothly until a characteristic "pop" is felt as the needle passes through the dura (usually 4 to 5 cm into the skin).
13. The stylus will be removed to observe for fluid return.
14. 3 cc of CSF will be collected in two plastic labeled tubes.
15. The needle will be removed from the patient's back.
16. A sterile dressing will be placed on the site and the patientplaced in the supine position for 2 hours.

APPENDIX 5: CONSENT FORM EXPLANATION

SEROPREVALENCE OF CRYPTOCCOCAL ANTIGENEMIA IN HIV-POSITIVEADULTS ATTENDING THE KENYATTA NATIONAL AND MBAGATHI HOSPITALS

Purpose of the study

I Dr. Irene Muchiri am undertaking this study on seroprevalence of Cryptococcal antigenemia in the HIV positive patients in Kenyatta National and Mbagathi Hospitals medical wards. Cryptococcal antigenemia is a subclinical condition that may lead to Cryptococcal Meningitis, an infection in the brain, which is the 2nd most common opportunistic infection in HIV patients.

Procedures

You are being asked to participate in this study that will take about 30 minutes. If you agree to participate I will ask you to sign a consent form. There will be a series of questions that I will ask you in confidence and all your responses will be noted down. Most questions have a 'No or Yes' for an answer and will require you to remember some things in the past. I will also do a physical examination on you to look for any signs of Cryptococcal meningitis, obtain your weight and height and classify you according to the WHO staging for HIV disease.

Thereafter my assistant/or I will collect from a blood sample of about 2mls that will be for evaluation for CRAG serology, a marker of Cryptococcal infection.

The tests results will be revealed to you, your primary doctor and the results attached in the file for your continued care. Tests results shall remain confidential.

Risks to you as a participant

There will be some discomfort from the needle prick at the site of blood sample removal (*usually from the area above the elbow or any other appropriate site*)

Rarely swelling or bleeding may occur from the puncture site but I or my research assistant will make sure bleeding has stopped before we leave. In the event that bleeding appears, kindly contact me or any nearest heath worker for assistance.

Benefits

You will not be charged for any of the lab tests.

The findings of the physical examination and laboratory tests will form part of your usual care; you will be treated if found to be CRAG positive. Copies of the test results shall be availed to your healthcare provider in your file

This is the first time the study is being done in Nairobi Kenya in the HAART naïve HIV population and the findings may go a long way in helping both the patients and heath profession in terms of identifying ways of treating and prevention of the Cryptococcal disease.

Right to refuse

Your participation in this research is voluntary. You are free to withdraw from the interview at any time and you shall not be discriminated upon. You are free to ask any questions and have a right to satisfactory answers before you sign the consent form.

If you agree to participate in this survey may you kindly sign on the consent form?

Thank you

APPENDIX 6: MAELEZO YA IDHINI

Kwa majina naitwa **Dr IRENE MUCHIRI** , mwanafunzi wa shahada ya uzamili katika Idara ya Magonjwa ya Ndani(Internal Medicine) ya Chuo Kikuu cha Nairobi, nafanya utafiti kwa watu walio na viini vya cryptoccocus vinavyosababisha menengitis na walio na virusi vinavyosababisha ukimwi, na waliolazwa wodi katika Hospitali kuu ya Kenyatta na Hospitali ya Mbagathi.

Nia ya Utafiti.

Utafiti huu si wa kupeana tiba lolote ila ni wa kuangalia idadi ya watu walio na shida ya viini vya cryptococcus vinavyosababisha meningitis ambao wanaishi na virusi vya HIV katika wodi , hospitali kuu ya Kenyatta na hospitali ya Mbagathi.

Taratibu.

Kama unakubali kushiriki katika utafiti huu utaombwa:

1. Kujibu maswali kadhaa ya kijamii na ya kuhusu ugonjwa wako.
2. Kufanyiwa uchunguzi wa kimwili na kupimwa ratili na urefu.
3. Kutolewa mililita 3 za damu tupeleke kupima viini vya cryptococcus.

Hatari.

Kwa kushirikikatikautafitihuu, mgonjwahatakuwakwenyehatariyoyoteilatukutakuwa na maumivumadogo wakati wa kutoa damu.

Faida ya Kushiriki:

1. Uchunguzi wote utafanywa bila malipo yoyote kutoka kwako. Mpelelezi mkuu ndiye atakayegharamia uchunguzi wa maabara
2. Matokeo ya uchunguzi huu yatafafanuliwa kwako na nakala iwekwe katika faili yako , ya matibabu kwa ajili ya kutazamwa na daktari msingi katika kliniki.
3. Kwa wale walio na viini vya cryptococcus , daktari wa kliniki ataelezewa ili aanze matibabu

Usiri.

Nakala yoyote itakayotokana na huu uchunguzi itahifadhiwa kwa usiri na kutumiwa kwa ajili ya utafiti huu tu.

Hitimisho.

Kushiriki kwako katika utafiti huu ni kwa hiari yako na uko huru kutoka wakati wowote , katika kipindi hiki cha utafiti. Ukikataa kushiriki au utake kuondolewa kutokana na utafiti, haita adhiri kwa njia yoyote ubora wa matibabu yako.

Kwa maelezo au maswali yoyote kuhusu utafiti huu, unaweza kuuliza:

Dr Irene Muchiri
Mchunguzimkuu,
Nambariyasimu 0728533716
Idara ya Magonjwa ya Ndani(Internal Medicine)
Chuo kikuu Cha Nairobi.

The Chairman of the Ethical and Review committee

Kenyatta National Hospital

Tel 020-2726300/0722-829500/0733-606400 Ext, 44102

APPENDIX 7: CONSENT FORM

I……..consent to participate in the study on THE SEROPREVALENCE OF CRYPTOCOCCAL ANTIGENEMIA IN THE HIV PATIENTS AT THE KENYATTA NATIONAL AND MBAGATHI HOSPITALS. I do this with the knowledge of the purposes of the study and the procedures thereof. The purposes of the study and procedures have been explained to me clearly by DR. IRENE MUCHIRI or her assistant. I am also aware that I can withdraw from this study without losing any benefits and quality of care of my medical condition.

Signature of patient……Date……...

Signature of witness…….……Date……..

If you have any questions during the course of the study, you may contact the following.

Dr. Irene Muchiri

Mobile number 0728533716

OR

The Chairman of the Ethical and Review committee

Kenyatta National Hospital

Tel 020-2726300/0722-829500/0733-606400 Ext, 44102

APPENDIX 8: QUESTIONNAIRE

SEROPREVALENCE OF CRYPTOCCOCAL ANTIGENEMIA IN THE HIV ADULT PATIENTS IN KENYATTA NATIONAL AND MBAGATHI HOSPITALS

A) DEMOGRAPHIC AND CLINICAL DATA

i. Study serial Number [ ]
ii. Hospital number [ ]
iii. Consent , Interview Language
    a. Consent has been read and obtained? [ ]
iv. Date and time of interview [ ]
v. Contact phone number where possible [ ]
vi. Date of HIV diagnosis [ ]

1. When were you born? [ ]

2. Gender (*as observed*)

[ ] Male,

[ ] Female

3. Are you married?

[ ] No [ ] Divorced, [ ] Widowed [ ] Never married.

[ ] Yes

4.-Where do you reside?

[ ] Urban

[ ] Rural

[ ] Peri urban

5. What is you're occupation?

[ ] Formal

[ ] Casual

[ ] Farmer

B) History and physical examination

1. Headache (>2 wk.)

[ ] Yes

[ ] No

2. Fever

[ ] Yes

[ ] No

3. Neck stiffness

[ ] Yes

[ ] No

4. Altered level of consciousness

[ ] Yes

[ ] No

5. Seizures

[ ] Yes

[ ] No

6. Projectile vomiting

[ ] Yes

[ ] No

7 Photophobia

[ ] Yes

[ ] No

C) What symptom does the patient present with? (Opportunistic infection)

D) WHO staging

[ ] Stage 1

[ ] Stage 2

[ ] Stage 3

[ ] Stage 4

E) Laboratory Measures

1. CD4 count cells/µL [ ] Enter value

2. Serum CRAG serology [ ] Positive [ ] Negative

3. Results of the CSF CRAG [ ] Positive [ ] Negative