An assessment of the use of viral load as a marker for Cryptococcal Antigen screening among HIV positive patients at Jaramogi Oginga Odinga Teaching and Referral Hospital

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Abstract- Cryptococcal meningitis, one of the leading opportunistic infections among HIV-infected persons with advanced disease, has a mortality rate of 67%. Cryptococcal Antigen (CrAg) test detects *Cryptococcus neoformans* antigen threeweeks before onset of symptoms when institution of therapy is likely to improve patient outcomes. Previously, CD4counts were used for ART monitoring and patients were only flagged for a targeted viral load test (VL) to confirm suspected treatment failure if there was a 30% CD4 decline from peak or failure to increase CD4 counts to 100 cells/µl after 12 months of therapy. At that time, patients were eligible for CrAg screening when CD4 counts were <100 cells/µl. Currently bi-annual HIV viral load measurements (VL) are used in ART monitoring and a patient is said to be failing therapy if two consecutive VL counts are ≥1000 copies/ml with institution of adherence and opportunistic infection treatment interventions between the two VL tests; CD4 tests are still be used in the differential diagnosis of some Opportunistic infections in symptomatic HIV-infected patients. A cross-sectional survey, riding on routine HIV program work, will be done to determine the prevalence of cryptococcalantigenemia and to assess the usability of VL as a marker for cryptococcal antigen testing in HIV-infected patients. We will perform CD4 quantification and CrAg test on blood samples for routine VL drawn from HIV infected patients at the JOOTRH HIV clinic. Laboratory results from these tests (CD4, CrAg, and VL) will be linked to the patients' medical records at the HIV clinic. Among patients who test CrAg positive, measures of validity will be computed for CD4 criteria against VL defined criteria. Subsequently, we will determine whether VL measurements can be used as an alternative to CD4 measurements. Then VL can then be used to direct when to screen for *C. neoformans.*

Index Terms- *Cryptococcus Neoformans, HIV/AIDS, HIV Viral Load, Screening*

I. INTRODUCTION

Cryptococcal meningitis (CM), is one of the leading opportunistic infections among HIV-infected persons with advanced disease[1]. Cryptococcal meningitis accounts for two thirds of all cases on adult meningitis among HIV infected patients in Africa[2 3] More than half of HIV infected patients with meningitis (from all causes) die within six months[4].The six-month survival rates for Cryptococcal meningitis 67.2%and two thirds of HIV infected patients with *Cryptococcus neoformans* (624700/957900) worldwide died in 2009[1 5-7]. Between 2011 and 2012, more than half of the patients admitted with Cryptococcal meningitis in South Africa died within 14 days (acute mortality); the risk of acute mortality was three times higher among those with a high cryptococcal burden denoting a delayed diagnosis [8]. CrAg positive patients in the US between 1986 and 2012 had a significantly shorter survival than CrAg negative patients (2.8 years vs. 3.8 years, p=0.03) [1].

The prevalence of Cryptococcal meningitis among HIV infected patients with CD4 <200 cells per microliter in Nyanza province in 2011 was 11% [9]. At the Jaramogi Oginga Odinga Teaching and Referral Hospital, prevalence rates of 18.5% have been reported among patients with CD4 <100 cells per microliter (Sigo, T, Laboratory Coordinator, JOOTRH, 20th December 2014Personal communication).More than 90% of patients who test positive for Cryptococcal Antigen, CrAg in cerebrospinal fluid also have a positive serum latex; serum CrAg test therefore provides a useful alternative for *C. neoformans* screening [10]. CrAg lateral test detects *C. neoformans* antigen 3 weeks before onset of symptoms and early institution of therapy prior to the onset of neurological deficit is associated with better outcomes[5 11].

Screening for CrAg has also been found to be cost effective. The prevalence threshold for which the benefits of Cryptococcal meningitis screening among HIV infected patients with CD4 <100 exceeded its costs was 2% and remained less than USD 140 per life saved where CrAg positivity prevalence was >2% [1]. Based on published data with CRAG prevalence rates of 2–12%, the cost to save one life was between $20 to $140 in Sub-Saharan Africa[12].

When computing cost effectiveness of CrAg screening using cost per life year gained, a measure that is more appropriate when mortality is high, the cost per life year gained for Cryptococcal meningitis screening in Vietnam where Cryptococcal meningitis prevalence was between 2-6% was within the WHO-CHOICE guidelines that state that these costs should not exceed the GDP per Capita[13 14].

For this reason, the WHO in 2010 recommended routine screening for *C. neoformans* in HIV-infected persons with advanced immunosuppression i.e. patients with CD4 <100 cells/µl.[1].In Kenya, this was adopted in 2011[15]. ART guidelines were been revised in July 2010 and in June 2014 to use the

following CD4 cut offs,350cells/µ and 500cells/µl respectively for ART initiation[15-18].Prior to November 2013, CD4 counts were used for monitoring all HIV infected patients regardless of ART status, after every six months or when deemed necessary by the clinicians; VL was selectively used where treatment failure was suspected; based on set eligibility criteria for HIV VL quantification which included a 30% CD4 decline form peak or failure to increase CD4 counts to 100 cells /µl after 12 months of therapy. Currently bi-annual HIV viral load measurements (VL) are used in ART monitoring and a patient is said to be failing therapy if two consecutive VL counts are ≥1000 copies/ml with institution of adherence and opportunistic infection treatment interventions between the two VL tests[15].

However, in 2014, ART guidelines were revised; the CD4 cell count threshold for ART initiation was increased to 500 cells per microliter and routine viral load was instituted in place of CD4 for ART monitoring.HIV-infected patients not on ART would continue to be monitored bi-annually using CD4 and would be initiated on ART when their CD4 cell count is<500 cells/µl[18].Due to this, it would be unlikely to find patients with CD4 cell counts of <100 cells/µl; this is because at a CD4 of 500 cells/µl all patients would be initiated on ART after which only VL (and not CD4) would be used for laboratory monitoring. No further guidelines were provided to guide screening for*C.neoformans*using CrAg[15].

However, CD4 tests could still be used in the absence of VL measurements and to assist in the differential diagnosis of some opportunistic infections in patients who present with new signs and symptoms regardless of ART status. This implies that CD4 measurements can only be done late in the course of an illness when the patient is symptomatic and the prognosis of CM is likely to be poor [10].

VL quantification could be used as an alternative for CD4 measurements in determining when to screen for cryptococcal infection. However, there is a poor correlation between VL and CD4 measurements[15 19]. Consequently, clinicians have no clear guidelines on when to screen for*C. Neoformans*infections.Therefore this study aims to determine the whether VLcan be used as a marker forCrAgscreeningamong HIV infected patients.

B.Null hypothesis

A Viral Load of 1000or more copies in a Human Immunodeficiency Virus infected person is not associated with the presence of Cryptococcalantigenemia

C. Study Objectives

General Objective

To determine the prevalence of Cryptococcal meningitis and assess the usability of Viral Load as a marker for Cryptococcal Meningitis testing in Human Immunodeficiency Viruspositive patients

Specific Objectives

1. To determine the prevalence of Cryptococcal Meningitis among Anti-retroviral Therapy experienced Human Immunodeficiency Virus infected patients (who have been on ART for between 6 months to one year) at Jaramogi Oginga Odinga Teaching and Referral Hospital in 2015
2. To describe factors predictive of positive Cryptococcal Antigen tests in Anti-retroviral Therapy experienced Human Immunodeficiency Virus infected patients Jaramogi Oginga Odinga Teaching and Referral Hospital in 2015
3. To determine the viral load cut off levels for a Cryptococcal Antigen test recommendation in ART experienced Human Immunodeficiency Virus infected patients Jaramogi Oginga Odinga Teaching and Referral Hospital in 2015.
4. To assessthe correlation between viral load and Cluster of Differentiation 4 measurements among ART-experienced HIV infected patients Jaramogi Oginga Odinga Teaching and Referral Hospital in 2015

II. METHODOLOGY

A.Study design and setting

The study seeks to ride on existing and ongoing routine HIV program work. Blood samples (for VL/CrAg and CD4) will be obtained from that already drawn from clients on their routine appointments in the HIV clinic. We will conduct a chart review for patients enrolled in HIV clinic in order to obtain required demographic and clinical data. This will be a cross-sectional study.

B.Study site

The is a prospective cross-sectional study will be conducted at JOOTRH HIV clinic and laboratory, the largest public health facility in the former Nyanza province and at KEMRI HIV Research laboratory. JOOTRH is located in Kisumu East sub-county, Kisumu County. It serves a catchment population of approximately 500,000 both from within the former Nyanza province and the neighboring provinces. It hosts the premier HIV clinic in the county. The JOOTRH HIV clinic was incepted in 2003 and has a cumulative patient enrolment of approximately 22,000; currently 5900 patients are actively accessing HIV management services in the clinic. Of the total active patients 64% of them are female, 95% of all active patients are aged 15 years and above and 85% of all active patients are on ART. Blood samples from HIV infected patients are usually collected at the JOOTRH lab.The VL tests are usually conducted at the KEMRI HIV Research laboratory.

C. Study population

HIV infected ART experienced patients (who have been on ART for between 6 months and one year) currently enrolled at the JOOTRH HIV clinic

i. Criteria for inclusion of subjects

- All HIV infected patients who regularly attended to at the JOOTRH clinic who have been on ART for at least 6 months and at most one year and whose samples have been collected for routine VL measurements
- Children aged less than 18 months of agewho have been confirmed positive using a positive HIV DNA PCR testto and have been on ART for at least 6 months and at most one year [20]

ii. Criteria for exclusion of subjects

- All HIV-infected patients attending the clinic who have been on ART for less than six months(e.g. children aged less than 6 months of age) and more than one year
- All samples that are rejected using routine laboratory rejection criteria e.g. Patients whose samples that are not well collected e.g. inadequate samples, clotted samples, leaked samples, samples transported in inappropriate temperatures and those that have passed the testing time limits.
- Samples from persons who are inebriated, unaccompanied minors (aged <18 years) or persons who cannot give consent for any other reason

C.Sample size calculation

Sample size was computed using the Buderer's formula for diagnostic studies in order to be 95% confident that the sensitivity of the Criteria is within plus or minus 5% of the population estimated sensitivity:

Sample size (n) based on sensitivity= $\frac{Z^2_{1-\alpha/2} \times S_N \times (1 - S_N)}{L^2 \times \Pr evalence}$

Sample size (n) based on specificity= $\frac{Z^2_{1-\alpha/2} \times S_P \times (1 - S_P)}{L^2 \times (1 - \Pr evalence)}$

Where

n = required sample size,

S_N= anticipated sensitivity,

S_P= anticipated specificity,

α = size of the critical region (1 − α is the confidence level),

$Z_{1-\alpha/2}$ = standard normal deviate corresponding to the specified size of the critical region (α),

L = absolute precision desired i.e. level of accuracy

The prevalence chosen should be selected with caution , lest the sample size adopted results in a loss of precision or confidence or both [2].The prevalence of Cryptococcal meningitis among patients with CD4<100 cells per microliter, ranges from 2.9% in the United States to 21% in Nigeria[5]. We estimated the prevalence of the CM in our target population in Kisumu based on the Nigeria study which is somewhat similar to our setting. Since our study enrolls only patients who have been on ART for at least 6 months, we adjust the prevalence rate accordingly. The adjustment factor is based on the proportion of patients, from our data, who initially had a CD4 count of < 100 but afterwards increased above 100 at 6 months or more after enrolling for ART. That brings the estimated prevalence to 12.6%.

Since the sensitivity and specificity of viral load cut-off point on when to screen for CrAg is unknown, we used an estimate of 50%; a conservative choice that protects the precision of the maximum width. The standard normal deviate corresponding to 95% confidence interval is 1.96 while the absolute precision desired, L, is 0.1 [1].

Implementing the formulas above in a SAS (SAS Institute Inc., Cary, NC) program yielded

$$n(sensitivity)=763$$

$$n(specificity)=110$$

Ideally, the preferred sample size that will give a precision of 0.1 or less for both sensitivity and specificity is 763.

Hence a total of 763 participants should be screened in order to detect approximately 29patients with CM. Five percent of all participants will be aged less than 15 years of age in conforming to our target population.

E.Study Procedures

Routine blood draw and preparation for Viral load testing

Currently, the clinicians at the JOOTRH HIV clinic routinely identify patients who have been on ART for between 6 months to one year and refer them to the HIV clinic lab for blood draw. At the JOOTRH lab, a laboratory technologist collects 4ml (4000 µl) of venous blood from the forearm in an EDTA tube from each patient. The sample is centrifuged and 2100 µl plasma is harvested. Of this, 1800 µl is sent to the KEMRI HIVResearchLaboratorywhere viral load testing for HIV infected patients in the former Nyanza province is routinely condutcted. The extra plasma (300 µl) and cells sediment (1900 µl) is then discarded.

Study-specific procedures

Participant recruitment

Participants will be recruited by a study assistant, from patients who have been sent for routine viral load by their attending clinician, at the JOOTRH lab. This will be done after blood draw for routine viral laod. Before they leave, participants will be informed that the study wishes to conduct additional testing on the same blood sample and to access their medical records at JOOTRH. Should participants wish to join the study, written informed consent will be obtained from adults and mature minor; assent will be obtained from persons aged 13-17 years and parent permission from participants aged less than 13 years. We will verify parent-child or guardian-child from the HIV clinic community department who conduct home visits at enrolment into HIV care. Participants notified of when and how their results will be sent to their attending clinician. Should they decline, their samples will be processed for viral laod as indicated above. Clinical and demographic characteristics of those who decline will be compared to those who accept participation; this is because if they differ significantly, this may bias our results.

Study specific laboratory tests that will be conducted on routine blood drawn for viral load sampling

In this study, at the JOOTRH lab50 µl of whole blood will be pipetted from the 4000 µl sampleof already drawn venous bloodand used for CD4 determination prior to centrifugation. From the remaining blood sample (3950 µl), 2000 µl of plasma will be harvested; 1800 µl of plasma will then be sent for viral load testing and the rest (200 µl) will be used for CrAg testing.

Selection of study participants

Since 20% of HIV infected patients have CD4 counts less than 100 cells/µl at JOOTRH, we will recruit 80% of our study populationfrom the proportion of the patients of ART experienced who had baseline CD4 less than 100cells/ µl.This is because on average, CD4 counts increase at a rate of 50 cells per µl every 48 weeks[21]. For this reason, our sample will be limited to patients who have been on ART for between 6 months to one year.

Assignment of Study Identifiers

Patients will be assigned study IDs after providing written informed consent for adults and mature minors; for minors, parental/guardian permission for children aged less than 13 years of age and assent for children aged 13-17 years will be sought. A link log that contains patient names and study Identifiers will be kept in a locked cabinet by the Principal investigator. Only study IDs will be used to label study documents; no participant names shall be indicated on study samples or forms. These study IDs will be indicated on the viral load log book and on all study samples; study samples will also be labeled with PSC numbers.The viral load log book also contains patient identifiers like patient names and Patient support Center (PSC) numbers which will assist in linking participant laboratory results to clinic information.

Transportation of samples from JOOTRH lab to KEMRI HIV Research Laboratory for VL testing

A transportation log (in Duplicate) will be used to track study samples that are sent to the KEMRI HIV Research Laboratory. This will be signed at the JOOTRH lab and at the receiving lab (KEMRI HIV Research Laboratory).

Obtaining VL results

Viral load results will be sent by electronic mail to the JOOTRH CRC data office from where they will be disseminated (print and electronic copies) to JOOTRH HIV clinic. Print copies received at the clinic will be sorted; results manually entered into an electronic VL database and the hard copy will then be filed in the patient's medical records at the HIV. Electronic copies will be sent to a laboratory officer, a clinician and a data manager from JOOTRH. Results from Viral load will be obtained using the usual results transmission network (print or electronic).

Obtaining CD4 and CrAg test results

The CrAg results and CD4 results will be produced in duplicate. The original copy will be sent to the patient's file and the duplicate copy will be filled and stored in the laboratory file cabinet. All laboratory data will be stored in an electronic register (Laboratory Information Management System).CrAg results and CD4 results will be entered into the study database by the study assistant/phlebotomist.

F.Data management

Accessing the participants medical records

Patient details (clinic numbers, patient names, ages, gender) obtained from the laboratory database will be used to link laboratory information to HIV clinic databases which contain existing clinical patient information.

Data entry

At the end of each day, a data clerk will enter the study participants' data from the viral load log book. The results of CD4 tests, Crag tests and VL will be entered as soon as they are received into a Microsoft Excel database. Data clerk stationed at the JOOTRH laboratory will make follow up phone calls (and if necessary trips) to the KEMRI HIV Researchlaboratory to track test results that have been not been received within 4 weeks.

Datastorage

Data obtained from all study forms and registers will be entered into a Microsoft Excel database in a password protected computer. The usual data protection mechanism provided for in the laboratory and HIV clinic will be relied upon to safeguard the data. To ensure data validity, 10% of all entered records will be counterchecked with the source forms.

Data analysis

Demographic and clinical characteristics of the study participants will be summarized using descriptive statistics. Categorical variables will be summarized using frequencies and percentages while continuous variables will be summarized using mean and standard deviation or median and the corresponding inter quartile range. Test for associations between categorical variables will be conducted using Pearson's Chi Square test while the test of association between continuous variables will be conducted using a two sample t-test if they are normally distributed. If the continuous variables are skewed then we will use a two sample Wilcoxon rank sum test. Normality tests will be conducted using Shapiro and Wilks normality test.

To examine the correlation between CD4 and VL measurements, we will first plot the two variables using a scatter plot to assess the possible existing relationship between the two variables. Pearson correlation coefficient with its associated p-value will then be used to determine whether CD4 and VL measurements are statistically correlated. Simple linear regression will be fitted to answer the question of how we can predict CD4 count of a patient given the VL measurement. A locally weighted regression (lowess fit) will be fitted, as well, to examine if there is convincing evidence that the relationship between CD4 and VL measurements is not linear.

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) with their 95% confidence intervals (CI) will be computed to determine the viral load cut-off levels for a CrAg test recommendation in HIV infected patients

Logistic regression analysis will be used to identify potential factors independently and jointly associated with CrAg positivity for ART-experienced HIV infected patients. The pre-supposed potential factors are baseline CD4 count, duration art and viral load. The odds ratios and the corresponding 95% confidence limits will be reported. Statistical analysis will be performed using Stataversion 13.1 (StataCorp, College Station, Texas, USA) and p-value of less than 0.05 will be used to define statistical significance.

G. Ethical considerations

Voluntary participation

All HIV infected patients who are sent to the laboratory for viral load testing will be invited to participate in the study. Participation in the study is on a voluntary basis; potential participants may opt out at any time without affecting their chances of receiving routine health services at the hospital.

Confidentiality of participant information

All participants will be assigned a unique identity number which will subsequently be used in all study documents to protect participants' privacy. The link log that contains this information will be stored in a locked cabinet in a separate location only accessible to the PI.The data collection team will be bound by signed confidentiality agreements.

Completed forms will be stored in a locked cabinet accessible to only key project staff. Once paper records are no longer considered active they will be packed into document storage boxes and labelled accordingly. Computer files and data will be removed from the computer hard drives at the completion of the project. They will be stored on compact disks or magnetic tape with corresponding paper records in a secure locked cabinet in the records room.

Completed data collection tools and databases will have different disposition procedures but will be linked to each other when archived and will be made available only to the study team personnel who require this information to use for analysis. Paper records, files and data will be stored at KEMRI for three to five years after the last publication of the results. Final reports will be permanently archived at KEMRI.

Institutional Review Board oversight

The study will only commence after approval from KEMRI SERU and any other additional IRBs. Study procedures will be conducted within the mandates of the regulatory bodies.

III. DISCUSSION

A.Potential risks to participants

There is no direct risk subjected to the participants by this study since we are utilizing blood that has already been drawn for other purposes and no extra blood is required to conduct additional testing.Patients who do not wish to be part of the study will receive VL measurements and clinical care as per the current ART guidelines. There is the minimal risk of loss of privacy in the inadvertent divulgence of medical records; however, several measures will be instituted to mitigate this (see section on participant privacy).

B.Potential benefits to study participants

No additional sample will be collected; however, participants will receive additional tests for *C. neoformans* on the same sample utilized for VL testing. Additionally, HIV infected patients will benefit from evidence-based screening for *C. neoformans*, early diagnosis, institution of therapy and better prognosis. This is because, currently, clinicians are unsure of when to screen for CrAg since CD4 counts are no longer being used for routine ART monitoring. By the time a patient is suspected to have an OI (ie when he is symptomatic) the disease would be at an advanced stage when the prognosis is likely to be poor. For this reason, screening for CrAg in the absence of symptoms is beneficial to the patients.

C.Study Limitations

Patients who decline participation in the study may differ from those who accept to participate in this study. To mitigate this, we will collect minimal demographic and clinical information from their patient databases in order to see how they differ from those who accept to participate in this study. The use of routinely collected data at the HIV clinic is covered under SSC 1525.

D.Expected application of results

Results from this study willdetermine whether VL measurements can be used as an alternative to CD4 measurements in determining when to screen for *Cryptococcus neoformans*. If this is possible, ultimately it will guide clinicians and HIV programs in screening of HIV infected patients for *C. neoformans* in the absence of routine CD4 monitoring. HIV infected patients will benefit from timely diagnosis and initiation of therapy and will subsequently have improved outcomes when diagnosed with cryptococcal meningitis. In the event this study yields favorable results (i.e. Viral load results could potentially be used as a marker for CrAg testing) , we propose the scale up the study to a larger population to provide more robust findings.

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