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SENSITIVITY AND SPECIFICITY OF CRYPTOCOCCAL ANTIGEN LATERAL FLOW ASSAY FOR THE DIAGNOSIS OF HIV-ASSOCIATED CRYPTOCOCCAL MENINGITIS IN WESTERN KENYA
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SENSITIVITY AND SPECIFICITY OF CRYPTOCOCCAL ANTIGEN LATERAL FLOW ASSAY FOR THE DIAGNOSIS OF HIV-ASSOCIATED CRYPTOCOCCAL MENINGITIS IN WESTERN KENYA

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ABSTRACT

Background: **HIV-associated cryptococcal meningitis carries a high case-fatality-rate in sub-Saharan Africa. Diagnostic delays partly contribute to this. Rapid point-of-care tests may facilitate speedy diagnosis. This study aimed to determine the sensitivity and specificity of urine, serum and cerebrospinal fluid (CSF) cryptococcal antigen lateral flow assay for the diagnosis of HIV-associated cryptococcal meningitis compared with the gold standard CSF culture.**

Methods: **A cross-sectional study was conducted in the medical wards of Moi Teaching and Referral Hospital, Eldoret, Kenya. Adult (≥18years) HIV-infected in-patients suspected to have meningitis had paired samples of urine, serum and CSF collected and tested real time using the cryptococcal antigen lateral flow assay (rapid point of care test). CSF cultures were also conducted. Data were analyzed using STATA ® (Statacorp Texas USA®). Descriptive statistics were used to summarize demographic, clinical and laboratory parameters. Sensitivity and specificity of the rapid test were calculated with the CSF culture as the gold standard.**

Results: **Of the 302 participants included 172 (57%) were female, median age 37 years (IQR 30-45). The median $CD4^+$ cell count was 183/ul (IQR 54-333). Among 288 participants with available CSF culture results, 50(17%) had culture-confirmed cryptococcal meningitis. Urine rapid test had a sensitivity and specificity of 86 %(95% CI 73-94) and 95.7% (95% CI 92-98) respectively. Serum rapid test had a sensitivity and specificity of 92% (95%CI 81-98) and 94.9% (95%CI 91-97) respectively. CSF rapid test had a sensitivity and specificity of 92% (95%CI 81-98%) and 94.5% (95% CI 91-97) respectively.**

Conclusion: **Serum and CSF cryptococcal antigen lateral flow assay are highly sensitive and specific for the diagnosis of HIV-associated cryptococcal meningitis. Urine is relatively sensitive and specific. Serum and CSF cryptococcal antigen lateral flow assay can be used as a less expensive alternative to cryptococcal antigen latex agglutination method. Urine cryptococcal antigen lateral flow assay could be adopted as a rapid point of care diagnostic test in primary care clinics in low income settings without experience in handling CSF or serum, to fast track diagnosis of cryptococcal meningitis.**

INTRODUCTION

There are an estimated 36.7 million people living with the human immunodeficiency virus (HIV) in the world, majority of whom (70%) live in the sub-Saharan Africa.[1] Although antiretroviral coverage has expanded to cover 13.6 million of the HIV-infected population in sub-Saharan Africa, HIV-associated mortality remains higher than in the developed world. [2] Contributors to the high mortality include opportunistic infections, chiefly tuberculosis and cryptococcal meningitis. [3],[4]

Cryptococcal meningitis affects an estimated 967,000 individuals and is responsible for 624,000 deaths annually. Majority of these infections occur in sub-Saharan Africa where an estimated 720,000 individuals are infected and 504,000 deaths occur annually. [4] In Kenya, mortality rates of 30-40% have been reported for cryptococcal meningitis. [5] Contributors to the high mortality associated with cryptococcal meningitis include both a delay in patient presentation and a delay in diagnosis. [6],[7] Standard diagnostic tests for cryptococcal meningitis such as cerebral spinal fluid (CSF) culture, Indian ink staining and serological tests such as cryptococcal antigen latex agglutination are largely unavailable in many low income settings. [8] In addition, these methods are quite expensive and have a long turn-around time. Quicker diagnostic tests are required to fast track the initiation of treatment this lethal disease.

Recently, a rapid point-of-care cryptococcal antigen lateral flow assay (CRAG LFA) test has been developed to address the diagnostic gap that exists.[9] This test has a rapid turn-around time (10 minutes), is less costly and does not require special laboratory infrastructure nor specialized training. [10] However, the performance of this test has not been well evaluated in the usual clinical setting in the sub-Saharan Africa. The aim of this study was to determine the sensitivity and specificity of CRAG LFA for the diagnosis of HIV-associated cryptococcal meningitis when carried out in the usual clinical setting by clinicians without laboratory training. We hypothesize that CRAG LFA will have acceptable specificity and sensitivity compared to the gold standard CSF culture.

METHODS

Study design: this was a cross-sectional study

Study site: The study was conducted at the Moi Teaching and Referral Hospital (MTRH) in Eldoret, Kenya. MTRH is the second national referral hospital in Kenya and serves a catchment population of 16 million people (40% of Kenya's population), approximately 360,000 of whom are HIV-infected (national HIV prevalence of 5.6%).[11] The total bed capacity at MTRH is 720 with 96 beds in two adult general medical wards (bed occupancy 120-140%). Monthly morbidity data indicate that an estimated 40- 50% of all adult general

medical ward bed occupancy is by HIV-infected patients. Cryptococcal meningitis is the most common form of meningitis diagnosed here accounting for an estimated 46% of confirmed meningitis cases. (David Lagat et al, unpublished data)

Study population: The study included adult (≥ 18 years) HIV-infected in-patients at MTRH presenting with clinical features of meningitis.

Eligibility, sampling and recruitment

Participants were included if they were ≥18 years, HIV-infected and had a syndrome consistent with meningitis. Meningitis was defined as presence of a combination of any three of the following signs and symptoms: headache, neck pains, neck stiffness, visual disturbance, fever, convulsions and altered mental status. Participants were excluded if they had been on any systemic antifungal therapy containing Amphotericin B, fluconazole, flucytosine or any other systemic antifungals at doses used for the treatment of cryptococcal meningitis for more than 2 weeks. Participants with history of treatment for confirmed cryptococcal meningitis within the past 6 months were also excluded. Individuals meeting the eligibility criteria were consecutively identified and recruited from the adult general medical wards by the investigator.

Sample size: The sample size was calculated by a formula advanced by Buderer et al.[12] A minimum sample size of 247 participants was required to determine the sensitivity and specificity of CRAG LFA compared to CSF culture

Study procedures: Consented participants underwent lumber punctures and had paired samples of CSF, blood and urine collected. Each CSF sample was divided into 2 portions. One portion was sent to the central laboratory for CSF cultures. Blood samples were allowed to clot and the serum samples were then used for the tests. Portions of CSF, serum and urine were tested at the bedside using the rapid point of care CRAG LFA test by the clinician. Both the clinician and laboratory staff were masked to the results of one another. The results of the rapid tests were communicated to the primary physician who made decisions regarding treatment.

Sources of data: Data were collected into preformed data collection forms from medical charts, laboratory records and participant interviews.

Data analysis: Data were analyzed using Stata version 12.0 (Statacorp Texas USA ®). Demographic, clinical and microbiological characteristics of the study participants were summarized using descriptive statistics. Categorical variables were summarized as frequencies and percentages while continuous variables were summarized as means (standard deviations) or medians (inter quartile ranges). Sensitivities and specificities of urine, serum and CSF CRAG LFA were computed using CSF culture as the reference standard. Positive and negative likelihood ratios (LR^+ LR^-) were used to assess the clinical value of the tests. Criteria for determining the usefulness of a test based on LRs proposed by Jaeschke et al was adopted.[13] For instance, $LR^+ > 10$ indicates good value of a test in increasing certainty about a positive diagnosis while $LR^- < 0.1$ indicates good value of a test in increasing certainty about a negative diagnosis. Missing data were excluded from the analysis.

RESULTS

Among the 302 participants included, 57% were female, median age 37 years (IQR 30-45). The median $CD4^+$ cell count was 183 (IQR 54-333). As illustrated in **Table 1,** an estimated 53% of participants were taking antiretroviral

therapy. The most common presenting symptoms were headache, fever and altered mental status affecting 86%, 68% and 54% of participants respectively.

Out of 288 participants with CSF culture results, 50(17%) had culture-confirmed cryptococcal meningitis. **Table 2** is a cross tabulation of urine CRAG LFA and CSF culture. Compared to CSF culture (reference standard), urine CRAG LFA had a sensitivity of 86 % (95% CI 73-94%) and a specificity of 95.7% (95% CI 92-98%) in the diagnosis of HIV-associated cryptococcal meningitis. The overall accuracy of urine CRAG LFA was 94% (95%CI 91-96%), with a positive predictive value (PPV) and negative predictive value (NPV) of 90% (95% CI 86-93%) and 94% (95% CI 92-95%) respectively. The positive and negative likelihood ratios (LR^+ and LR^-) of urine CRAG LFA was 20 and 0.15 respectively, indicating that the test is of good discriminative value in the diagnosis of cryptococcal meningitis.

The cross-tabulation of serum CRAG LFA and CSF culture is shown in **Table 3.** Serum CRAG LFA had a sensitivity and specificity of 92% (95%CI 81-98%) and 94.9%(95% CI 91-97%) respectively compared to CSF culture. The overall accuracy of the test was 94.4% (95%CI 91-97%). The PPV and NPV of the test was 88.5% (95%CI 84-91%) and 96.5% (95% CI 95-98%) respectively. The LR^+ and LR^- of serum CRAG LFA was 18 and 0.084 respectively.

Compared with CSF culture (**Table 4)**, CSF CRAG LFA had a sensitivity and specificity of 92% (95%CI 81-98%) and 94.5% (95% CI 91-97%) respectively. The overall accuracy of the test was 94.1% (95%CI 91-97%) with a PPV and NPV of 87.8% (95% CI 84-91%) and 96.5% (95% CI 90-100%) respectively. The LR^+ and LR^- ratio for predicting presence of disease by CSF CRAG LFA was 16.7 and 0.08 respectively. Of 60 participants with positive CSF CRAG LFA, 60 (100%) and 50 (83%) had positive serum and urine CRAG LFA respectively. Other diagnostic characteristics of all the test methods are summarized in **Table 5.**

Table 1

Demographic and clinical characteristics of patients with meningitis at Moi Teaching and Referral Hospital (MTRH)

Variable	**N=302**	**Median (IQR) or proportion%**
Age (years)	302	37 (30-45)
Gender	302	
Male	130	43 %
Female	172	57%
$CD4^+$ cell count	41	183 (54-333)
ART status	302	
ART naïve	114	38%
ART<3months	46	15%
ART≥3months	115	38%
Missing	27	9%

Table 2

Cross tabulation of urine cryptococcal antigen lateral flow assay (CRAG LFA) versus cerebral spinal fluid (CSF) culture

	Urine CRAG LFA		
CSF culture	**Negative**	**Positive**	**Total**
Negative	225	10	235
Positive	7	43	50
Total	232	53	285

Table 3

Cross tabulation of serum cryptococcal antigen lateral flow assay (CRAG LFA) versus cerebral spinal fluid (CSF) Culture

	Serum CRAG LFA		
CSF culture	**Negative**	**Positive**	**Total**
Negative	223	12	235
Positive	4	46	50
Total	227	58	285

Table 4

Cross tabulation of CSF CRAG LFA versus cerebral spinal fluid (CSF Culture)

	CSF CRAG LFA		
CSF culture	**Negative**	**Positive**	**Total**
Negative	224	13	237
Positive	4	46	50
Total	228	59	287

Table 5

Summary diagnostic characteristics for different test methods for cryptococcal meningitis using cerebral spinal fluid (CSF) culture as reference standard

	Urine CRAG LFA	Serum CRAG LFA	CSF CRAG LFA
Sensitivity %(95%CI)	86 (73-94)	92 (81-98)	92(81-98)
Specificity% (95%CI)	95.7 (92-98)	94.9 (91-97)	94.5 (91-97)
Accuracy	94 (91-96)	94.4(92-97)	94.1 (91-97)
PPV%(95%CI)	89.6 (86-93)	88.5 (84-91)	87.8 (84-91)
NPV%(95%CI)	94 (92-95)	96.5 (95-98)	96.5 (90-100)
LR+	20.21	18	16.7
LR$^-$	0.1462	0.0843	0.0846
DOR	138.2	214.3	197.4

Key

PPV: Positive predictive value
NPV: Negative predictive value
LR$^+$: Positive likelihood ratio
LR$^-$: Negative likelihood ratio
DOR: Diagnostic odds ratio

DISCUSSION

We found a relatively high sensitivity and specificity of urine, serum and CSF cryptococcal antigen lateral flow assay for the diagnosis of HIV-associated cryptococcal meningitis. These results are in-keeping with previous studies that assessed the performance of CRAG LFA in various samples. For instance, those that evaluated the sensitivity of CRAG LFA in urine found sensitivities ranging between 70-98%.[10, 14, 15] Most of these studies had small sample sizes ranging between 13-62 and therefore the estimate had wide variability. When combined together in a meta-analysis, Huang et al found a pooled sensitivity of 85% for urine CRAG LFA, which was very similar to what we found.[16] These studies did not have sufficient data to compute specificity of urine CRAG LFA.

In the category of serum CRAG LFA, our findings generally agree with those of previous studies. For instance, the meta-analysis by Huang et al that included 8 studies with data on serum CRAG LFA found both a sensitivity and specificity of 98%.[16] The 95% confidence interval of our estimate (81-98%) overlaps with that found in the meta-analysis. Our study shows that serum CRAG LFA has a slightly better sensitivity and an almost equal specificity to urine CRAG LFA in the diagnosis of HIV-associated cryptococcal meningitis. However, given the ease and non-invasive nature of obtaining a urine sample, urine CRAG LFA may have a role in establishing the diagnosis of cryptococcal meningitis in low income settings.

With regard to performance of CRAG LFA in cerebral spinal fluid, our study findings generally agreed with those of others. The meta-analysis above, which included 6

studies with data on CSF CRAG LFA, found both sensitivity and specificity of 99% with 95% confidence intervals overlapping with what we found in our study. CRAG LFA has several advantages which make it an attractive option for use especially in low income settings. These include being cheap, equipment free, requires minimal training and has no special storage requirements. Given the generally good test parameters in urine, serum and CSF, CRAG LFA may revolutionize care for cryptococcal meningitis even in remote settings by providing rapid results.

There are several strengths to this study. For instance, the study staff running the bedside CRAG LFA tests and laboratory staff performing CSF cultures were masked to the results of each other, therefore limiting bias. We also compared CRAG LFA to the gold standard test, CSF culture, to limit potential misclassification of cases that would arise if we used antigen based methods used in many previous studies. In addition, prospective collection of CRAG LFA data in the usual clinical setting, by persons without special training in laboratory medicine improves the generalizability of our findings to similar low income settings.

The study had a few limitations. First, we presumed that a positive urine CRAG LFA indicated presence of cryptococcal meningitis. However, it is possible that some cases of positive urine CRAG LFA indicated urinary cryptococcosis since cryptococcuria can occur as an isolated urinary tract infection or may persist within the prostate without causing meningitis. [17],[18], [19] This would have the effect of increasing our false positive rates. However, since all our participants presented with CNS symptoms, it is reasonable to assume that those with positive urine CRAG LFA indeed had cryptococcal meningitis. Another factor that could potentially increase our false positive CRAG LFA rate is enrolling participants with asymptomatic cryptococcal antigenemia without established cryptococcal meningitis. In this scenario, the symptoms of meningitis might be due to bacterial infections for instance. However, our false positive rate was quite low and thus our results are robust.

CONCLUSION

Our results show that serum and CSF cryptococcal antigen lateral flow assay are highly sensitive and specific for the diagnosis of HIV-associated cryptococcal meningitis. Urine cryptococcal antigen lateral flow assay is relatively sensitive and highly specific for the diagnosis of HIV-associated cryptococcal meningitis compared to CSF culture. We recommend that serum and CSF cryptococcal antigen lateral flow assay should be adopted for the diagnosis of HIV associated cryptococcal meningitis in low income settings where latex agglutination methods are not readily available. Urine cryptococcal antigen lateral flow assay should be considered part of rapid point of care diagnostic tests for HIV associated cryptococcal meningitis in low income settings with inadequate laboratory infrastructure, and without experience in handling CSF and serum samples Future studies to assess the utility of urine and serum cryptococcal antigen lateral flow assay among asymptomatic HIV infected patients with advanced immunosuppression are recommended to determine whether these tools can be used in interventional programs that aim to prevent development of clinical cryptococcal meningitis.

ABBREVIATIONS

CSF	cerebrospinal fluid
HIV	Human Immunodeficiency Virus
LR	Likelihood ratio
MTRH	Moi Teaching and Referral Hospital
NPV	negative predictive value
PPV	Positive predictive value

DECLARATIONS

Ethics Approval and Consent to Participate. The study was reviewed and approved by the Moi University/MTRH Institutional Research and Ethics Committee. Participants provided written informed consent prior to participating in the study. For participants with reduced levels of consciousness, consent was sought from their next of kin.

Competing Interests: The authors declare that they have no competing interests.

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AUTHORS' CONTRIBUTIONS

C.M conceived the study and participated in its design, data collection and analysis and drafted the initial manuscript. C.O participated in study design and drafting of the manuscript. W.I was involved in study design and co-ordinated laboratory assays. A.S was involved in study design and revision of the manuscript. All authors read and approved the final manuscript.

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