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Occurrence of cryptococcal antigenaemia among HIV- uninfected patients with stroke in Nigeria: a pilot case-control study

SA Fayemiwo[1,2,7], OB Makanjuola[1,2], F. Bongomin[3], J Nwaokenye[2], S Diala[5], M Tito-Ilori[4], R Akinyemi[4,5,6] and MO Owolabi[4,5]

Department of Medical Microbiology and Parasitology[1], College of Medicine, University of Ibadan and University College Hospital[2], Ibadan, Department of Medical Microbiology and Immunology[3], Faculty of Medicine, Gulu University, Gulu, Uganda, Department of Medicine[4], Centre for Genomic and Precision Medicine[5] and Neuroscience and Ageing Research Unit[6], Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan, Ibadan, Nigeria and Division of Infection[7], Immunity and Respiratory Medicine, School of Biological Sciences, University of Manchester, Manchester, M13 9PL, UK

Abstract

Background: Infections have been associated with stroke occasionally, but are not considered to be a direct cause. We aimed to compare the prevalence of cryptococcal antigenaemia in stroke patients compared to stroke-free controls.

Methods: Through the Stroke Investigative Research and Education Network (SIREN) project, adults (aged ≥18 years) with stroke confirmed by computed tomography (CT) scan or magnetic resonance imaging (MRI) (cases) and age-matched and gender-matched stroke-free controls were recruited from the communities in catchment areas of the cases. A simple random sampling was used to select blood samples of stroke patients and controls in a 1:1 ratio. Serum cryptococcal antigen (CrAg) tests were performed using CrAg Lateral Flow Assays (BIOSYNEX^{R} Crypto PS).

Results: Of the 100 HIV-uninfected patients (50 cases and 50 controls), majority 70 (70%) were males with a median age of 57 (range: 33-86) years. Thirty-six (72%) cases and 23 (46%) controls were hypertensive (p<0.001), while 6 (12%) cases and 14 (28%) controls were diabetic (p=0.059). Overall, CrAg was positive in 16% (n=16) of the participants; 10 (20%) among cases and 6 (12%) among controls (Odds ratio: 1.83; 95% confidence interval: 0.61-5.5; p=0.275). Overall, 13 deaths occurred, all among cases; death rates were similar between CrAg positive (2/10, 20%) and CrAg negative (11/40, 27.5%) stroke patients (p=0.628). Diabetes mellitus had a trend towards predicting a positive CrAg among cases (Odds ratios, 1.52 (95% CI: 0.97-2.4); p=0.069).

Conclusions: Cryptococcal antigenaemia appears to be more common in stroke patients compared to controls. Adequately powered studies are required to explore this observation.

Keywords: *Fungal infections, Cryptococcal antigenaemia, Stroke, Diabetes, Nigeria.*

Résumé

Contexte: Des infections ont été associées à des accidents vasculaires cérébraux occasionnellement, mais ne sont pas considérées comme une cause directe. Nous visions à comparer la prévalence de l'antigénémie cryptococcique chez les patients victimes d'un AVC par rapport aux témoins sans AVC.

Méthodes: Dans le cadre du projet Stroke Investigative Research and Education Network (SIREN), des adultes (âgés de 18 ans ou plus) avec un AVC confirmé par tomodensitométrie (TDM) ou imagerie par résonance magnétique (IRM) (cas) et appariés selon l'âge et le sexe Des témoins sans AVC ont été recrutés dans les communautés des zones de desserte des cas. Un simple échantillonnage aléatoire a été utilisé pour sélectionner des échantillons sanguins de patients et de témoins d'AVC dans un rapport 1: 1. Des tests d'antigène cryptococcique sérique (CrAg) ont été réalisés à l'aide de tests CrAg à flux latéral (BIOSYNEXR Crypto PS).

Résultats: Sur les 100 patients non infectés par le VIH (50 cas et 50 témoins), la majorité 70 (70%) étaient des hommes avec un âge médian de 57 ans (intervalle: 33-86). Trente-six (72%) cas et 23 (46%) témoins étaient hypertendus (p <0,001), tandis que 6 (12%) cas et 14 (28%) témoins étaient diabétiques (p = 0,059). Dans l'ensemble, la CrAg était positive chez 16% (n = 16) des participants; 10 (20%) parmi les cas et 6 (12%) parmi les témoins (rapport de cotes: 1,83; intervalle de confiance à 95%: 0,61-5,5; p = 0,275). Dans l'ensemble, 13 décès sont survenus, tous

Correspondence: Dr. S. A. Fayemiwo, Department of Medical Microbiology and Parasitology, College of Medicine, University of Ibadan, Ibadan, Nigeria. E-mail address: dayteet@yahoo.com

parmi les cas; les taux de mortalité étaient similaires entre les patients ayant subi un AVC CrAg positif (2/10, 20%) et CrAg négatif (11/40, 27,5%) (p = 0,628). Le diabète sucré avait tendance à prédire un CrAg positif parmi les cas (odds ratios, 1,52 (IC à 95%: 0,97-2,4); p = 0,069).
Conclusions: L'antigénémie cryptococcique semble être plus fréquente chez les patients victimes d'un AVC que chez les témoins. Des études suffisamment puissantes sont nécessaires pour explorer cette observation.

Mots clés*: Infections fongiques, antigénémie cryptococcique, accident vasculaire cérébral, diabète, Nigéria.*

Introduction

Central nervous system (CNS) infections caused by fungal agents are usually severe life-threatening infections with the highest-burden in sub-Saharan Africa [1]. *Aspergillus, Candida* and *Cryptococcus* species are the predominant fungal agents of fungal infections of the CNS especially in stroke patients [1,2]. Central nervous system (CNS) infections with *Cryptococcus* usually arise from a primary lung infection and present with meningoencephalitis with a high mortality rate [2,4]. *Cryptococcus* possesses several specific virulence factors, which grant the organism tropism for the CNS. The organism usually crosses the blood-brain barrier and survive within the CNS environment [5-7].

Patients with cryptococcal meningitis present with neurological features, mainly headache and alteration of mental status, and also fever, nausea and vomiting. Symptom onset to presentation is usually longer in non-Human Immunodeficiency virus (HIV) patients [5,8]. *Cryptococcus* spp. causes basilar meningitis thus vessels at the base of the brain may become covered with exudates, develop vasculitis and inflammation, vasospasm and thrombosis with cerebral infarction [8,9]. Most of the patients develop focal signs related to the formation of parenchyma brain cryptococcomas while others develop cerebral infarctions related to the inflammatory occlusion of small leptomeningeal vessels [8,10]. Chronic meningitis from cryptococcal infection may result in cerebral infarction presenting as stroke [10]. Multiple large vessel strokes resulting from cryptococcal meningitis emphasize the importance of considering fungal infections in the differential diagnosis of stroke [9]. Cryptococcal meningitis presenting as stroke has been reported in immunocompromised individuals by many researchers [10-12]. A recent study reported a strong association of Acquired Immune Deficiency Syndrome (AIDS) with intracerebral haemorrhages and also ischemic strokes [12]. It has been recommended that cryptococcosis of the central nervous system should also be considered as a differential diagnosis in AIDS patients presenting with stroke [10,13]. This situation may however also occur in immunocompetent persons. Recurrent strokes secondary to cerebral infarction may occur, and has been reported in immunocompetent persons following reactivation of latent infections [14]. Furthermore, headache and neck pain, the classic symptoms of cryptococcal meningitis may not be present in immunocompetent persons rather, they may present with malaise, lethargy, altered mental status, seizures, and stroke, making diagnosis difficult [9,14].

While the overall incidence of fungal infections implicated in causing a stroke is relatively low, little is known about stroke in relation to the cryptococcal infection in immunocompetent patients. The objectives of this study were to determine the prevalence and factors associated with cryptococcal antigenaemia in stroke patients and to compare cryptococcal antigenaemia rates among stroke and stroke – free control subjects

Methods

Study site and design

The current study is nested within the Stroke Investigative Research and Education Network (SIREN) project a multicentre, multidisciplinary, case-control study carried out in Ghana and Nigeria. One of the objectives of this large study was to evaluate the risk factors of stroke in blacks in sub-Saharan Africa and the detail of the project has been published previously [15]. Stroke cases were consecutive consenting (consent was obtained from next of kin in unconscious or aphasic patients) adults, aged $\geq$18 years, with a first clinical stroke within 8 days of current symptom onset or last seen without a deficit. Controls were consenting stroke-free adults (aged $\geq$18 years), from the communities in the catchment areas of the SIREN study hospitals where cases were recruited or recruited from the study hospitals. Stroke-free status was confirmed with the Questionnaire for Verifying Stroke-Free Status (QVSFS)(16). Controls were matched by age, sex, and ethnicity [17]. Ethical approval was obtained from the University of Ibadan/University College Hospital (UI/UCH) Ethics review committee (UI/EC/17/0250) and informed consent was obtained from all participants before recruitment.

Sample size and selection

A simple random sampling was used to select blood samples of 50 stroke patients and 50 controls from the study population in nested pilot case – control design. Inclusion criteria were stroke patients and controls who were HIV negative confirmed by double Enzyme-Linked Immunosorbent Assay (ELISA) HIV testing.

Laboratory procedure

Serum from study participants was tested for the presence of cryptococcal antigen (CrAg) with the CrAg Lateral Flow Assay (LFA) (BIOSYNEX^R Crypto PS, CE-marked in Europe), a point-of-care test for the diagnosis of cryptococcosis. The detection kits were simple, sensitive and qualitative latex test for the detection of CrAg as previously described [18]. The tests were performed and interpreted according to manufacturer's instructions. Quality control of the latex kits for the determination of the presence of the CrAg was considered. External positive and negative controls samples provided by the manufacturer were assayed along with each of the test serum samples analysed to ensure that the kits were functioning properly.

Data analysis

Data were entered and analysed using Statistical Package for Social Sciences (SPSS) software version 21.0. Continuous variables were summarized using mean and standard deviation while categorical variables were summarized using frequencies and proportions. Differences between continuous variables were tested with student's T-test, ANOVA or Mann-Whitney test as appropriate while categorical variables were compared using the Chi-Square test, and the level of significance was set at $p \leq 0.05$. The dependent variable in this study was the presence or absence of CrAg. Independent variables include age, gender, and marital status, level of education, socio-economic status, and religion. Others include smoking status, headache, seizures, neck stiffness, and presence of comorbidities (hypertension, diabetes and seizures), neurologic deficit, raised intracranial pressure (ICP), urinary tract infection (UTI) and diseases outcome.

Results

Demographic characteristics

One hundred HIV-uninfected patients (50 cases and 50 controls) constituted the study population. Majority of the participants were males (n=70, 70%). The mean age of the cases was 58 (SD = 12.7,) years, while that of the stroke-free controls was 57 (SD = 12.6,) (Table 1). Among the cases, male participants were 35 (70%) while, 44 (88%) of them were married. Among the controls, 34 (69%) were males, while 45 (90%) were married. Among cases, 22 (44%) had tertiary education while 44 (88%) were currently married. Most of the patients were overweight. The mean BMI among the cases was 28.3 ±7.0 versus 25.6±5.2 in the control group (P=0.442). Two (4%) of the cases were current cigarette smokers, while only 2 (2%) were in the control group. (P=0.603).

Frequency of cryptococcal antigenaemia

Overall, 16 (16%) participants tested positive for CrAg; 10 (20%) of cases tested positive, while 6 (12.0%) controls were positive for serum CrAg (Figure 1). Greater than 50% of those that were affected were within the age category of 41-59. Compared to the controls, cases were about two times more likely to test positive for CrAg. However, this was not statistically significant (OR: 1.83, 95%CI: 0.61-5.5, p=0.275).

Comparison of comorbidities and clinical presentations among cases and controls

Comparing other characteristics of comorbidities and clinical presentations among cases and controls, 36 (72%) of 50 versus 23 (46%) had hypertension (P<0.001); 6 (12%) versus 14 (28%) had diabetes mellitus (P=0.059). Among the cases with stroke, 25 (50%) had a headache, 3 (6%) had neck stiffness, 13 (26%) had raised intracranial pressure (ICP), 16 (32%) had vomiting, 13 (26%) of the cases died when compared with controls which had 0%, and they were all statistically significant (P<0.001). Hypertension, headache, neck stiffness, vomiting, raised ICP are all significantly higher among the cases than the controls (P<0.0001), (Table 2)

Distribution of the co-morbidities and neurological features by cryptococcal antigen status

Headache were present in about 60% (6/10) of cases that are CrAg positive when compared with the CrAg negative cases (19/40, 47.5%). Another neurological symptom that occurred more at the time of presentation among CrAg positive cases was raised intracranial pressure (ICP), as it occurred in 40 % (4/10) of them versus 22.5% (9/40) in CrAg negative cases.

However, seizures (2.5%, 1/40 vs 0.0%, 0/10), neck stiffness (5.0%, 2/40 vs 0.0%, 0/10) and vomiting (37.5%, 15/40 vs 1.0%, 1/10) were not statistically significant for positive CrAg test as these

Table 1: Demographic characteristics of the patients with stroke and the controls

Variable	All participants Frequency (%)	Case Frequency (%)	Control Frequency (%)	P-value
Demographics n=100	n=100	n= 50	n=50	
Gender				
Male	70 (70)	35 (70)	35 (70)	1.000
Female	30 (30)	15 (30)	15 (30)	
Age, median (range)	57.5 (33-86)	57.9 (33-86)	57.2 (33-81)	0.277
Age groups (years)				
<40	7 (7)	4 (8)	3 (6)	0.756
41-49	21 (21)	8 (16)	13 (26)	
50-59	27 (27)	15 (30)	12 (24)	
60-69	23 (23)	11 (22)	12 (24)	
>70	22 (22)	12 (24)	10 (20)	
Education	n=100	n= 50	n=50	
No formal education	2 (2)	1 (2)	1 (2)	1.17
Primary	21 (21)	5 (10)	16 (32)	
Secondary	37 (37)	21 (42)	16 (32)	
Tertiary	37 (37)	22 (44)	16 (32)	
Postgraduate	2 (2)	1 (2)	1 (2)	
Marital status	n=100	n= 50	n=50	
Never married	3 (3)	2 (4)	1 (2)	0.685
Currently married	89 (89)	44 (88)	45 (90)	
Separated	1 (1)	1 (2)	0 (0)	
Widow/Widower	7 (7)	3 (6)	4 (8)	
Tobacco use	n=100	n= 50	n=50	
Current smoker	4 (3)	2 (4)	2 (2)	0.603
Ex-smoker	6 (6)	2 (4)	4 (8)	
Never	89 (91)	46 (92)	43 (90)	
Body mass index	n=100	n= 50	n=50	
Mean BMI, SD	27.0±6.3	28.3 ±7.0	25.6±5.2	0.424

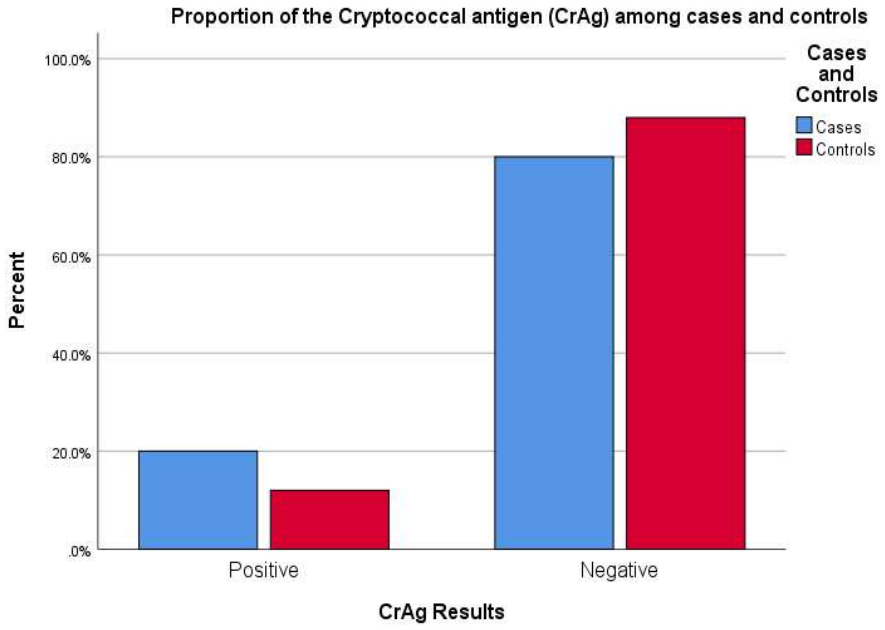

Fig. 1: Bar chart showing the proportion of the Cryptococcal antigen (CrAg) among cases and controls

Table 2: Characteristics of the patients' comorbidities and clinical presentations

Variable	All participants Frequency (%)	Case Frequency (%)	Control Frequency (%)	P-value
Co-morbidity				
Hypertension	n=100	n= 50	n=50	
Yes	59 (58)	36 (72)	23 (46)	<0.001
No	37 (37)	10 (20)	27(54)	
Do not know	4 (4)	4 (8)	0 (0)	
Diabetes	n=100	n= 50	n=50	
Yes	20 (20)	6 (12)	14 (28)	0.059
No	78 (78)	42 (84)	36 (72)	
Do not know	2 (2)	2 (4)	0 (0)	
Clinical presentation				
Seizure	n=100	n= 50	n=50	
Yes	2 (2)	1 (2)	0 (0)	0.315
No	98 (98)	49 (98)	50 (100)	
Headache	n=100	n= 50	n=50	
Yes	25 (25%)	25 (50)	0 (0)	<0.001
No	75 (75%)	25 (50)	50 (100)	
Neck stiffness	n=100	n= 50	n=50	
Yes	3 (3)	3 (6)	0 (0)	0.079
No	97 (97)	47 (94)	50 (100)	
Vomiting	n=100	n= 50	n=50	
Yes	16 (16)	16 (32)	0 (0)	<0.001
No	84 (84)	34 (68)	50 (100)	
*Raised ICP**	n=100	n= 50	n=50	
Yes	13 (13)	13 (26)	0 (0)	<0.001
No	87 (87)	37 (74)	50 (100)	
*UTI**	n=100	n= 50	n=50	
Yes	3 (3)	3 (6)	0 (0)	0.242
No	96 (97)	47 (94)	49 (0)	
*CrAg test**	n=100	n=50	n=50	
Positive	16 (16)	10 (20)	6 (12)	0.275
Negative	84 (84)	40 (80)	44 (88)	
Outcome	n=100	n=50	n=50	
Died	13 (13)	13 (26)	0 (0)	<0.001
Alive	87 (87)	37 (74)	50 (100)	

* ICP – Intracranial pressure, UTI- Urinary tract infection, CrAg- Cryptococcal antigen

Table 3: Predictors of cryptococcal antigen positivity

Variable	Adjusted OR (95% CI)	P-value
Hypertension	0. 97 (0.70 – 1.34)	0.845
Diabetes mellitus	1.52 (0.97 – 2.40)	0.069
Stroke cases	2.42 (0.71 – 8.3)	0.157

symptoms occurred more in CrAg negative cases than the CrAg positive cases (P>0.05). For the co-morbidities, being diabetic (20.0%, 2/10 vs 2.0%, 1/40) and having urinary tract infections (20.0%, 2/10 vs 2.0%, 1/40) were significantly associated with a positive CrAg test. Hypertension and current tobacco smokers were not associated with CrAg positivity (P>0.05).

Multinomial logistic regression was calculated to determine the risk of CrAg positivity in the patients following a multivariate model. Cases of stroke (Adjusted Ratios (AR) = 1.5 (95%CI: 0.97-2.40), hypertension and diabetes mellitus (AR= 1.5 (95%CI: 0.97-2.40) have an increased likelihood of testing positive to CrAg, but this did not attain statistical significance.

Discussion

Central nervous system (CNS) mycotic infections are associated with tremendous morbidity and mortality such that early recognition of neurologic syndromes with careful planning and implementation of efficacious management is of prime importance [4]. One-fifth of patients with cryptococcal disease may not show any known underlying immunocompromising conditions, but contribute up to 50% of the attributable mortality due to delays in the diagnosis of an unsuspected host [19,20]. As cryptococcal infections of the nervous system can result in severe neurological disability and also death, early recognition and prompt diagnosis are essential in improving the prognosis even when there is a low index of suspicion [14,21]. Cryptococcal antigen (CrAg) screening is routine in patients with advanced HIV disease and has been shown to be life saving and cost-effective [22]. However, in HIV-uninfected patients at risk of cryptococcal antigenaemia and cryptococcal disease, like those with liver cirrhosis and stroke patients – there is insufficient evidence to support routine screening.

In this study, overall, CrAg was positive in 16% of the participants, and close to similar proportions of the cases and controls were CrAg positive, although the antigenaemia prevalence was slightly higher among cases than their controls. This is not unexpected as cryptococcosis may present indolently in some of these immunocompetent host, causing the delays in diagnosis, and subsequent neurologic sequelae like stroke, cranial neuropathies and cognitive impairment. This result was in agreement with findings of Prasad *et al* [23], which had demonstrated that 16.6% of their patients were found to have cryptococcal meningitis. In another similar study, 43.65% of apparently immunocompetent patients had cryptococcosis [24]. Our finding was however higher than the prevalence of 8.3% obtained in a similar study among the immunocompetent patients in India [25]. Surprisingly however, our rate of 16% antigenaemia was higher than the findings from other studies among immunocompromised patients in Ethiopia (8.5%) [26], southern zones of Nigeria (11.4% - 13.1%) [18,27], and Senegal (1.2%) [28]. Our cryptococcal antigenaemia prevalence rate was also slight lower than the findings of 16.7% in another study among immunocompromised patients from northern part of Nigeria [29]. The global cryptococcal antigenaemia rate is 6% among patients with advanced HIV disease [30].

On the demographic characteristics, there is nearly equal male to female ratio in the cases and controls patients. This is expected as both sexes are subjected to the same level of environmental exposure. Majority of the patients with positive cryptococcal antigenaemia are also in the range of 50-69 years old. This must have been due to many years of indolent or latent infections, with the organism finding their way to cross the brain-barrier system. Non-outbreak strains of *Cryptococcus* species have been associated more with severe neurological complications. *Cryptococcal neoformans* and *C. gatti* are the leading causes of meningitis in both immunocompromised and immunocompetent hosts because of their tropism for dopaminergic tracts [24]. *Cryptococcus* species also pervade the perivascular space of the penetrating basal arteries and induce proliferation of the endothelial cells causing pan-arteritis, thrombosis, and occlusion [31]. This result is in agreement with the results of Lui, *et al* who reported the mean age of the immunocompetent patients with cryptococcal infection to be 52.8 ± 3.6 [24]. It would be nice to know the aetiology of the *Cryptococcus species*, as *C. gatti* are more associated in immunocompetent individuals. Our result is however contrary to the findings in the studies among immunocompromised hosts, especially HIV-infected patients which showed that the predominated age groups that are mostly affected are in the range of 31-45 years old [18,28,29].

Most of the non-HIV patients with cryptococcal infections had clinical presentation challenges like fever, neck stiffness and other meningeal signs. In a case previously published, a healthy man was diagnosed with depression, after many months in the hospital, the patient underwent a lumbar puncture to reveal concealed cryptococcal disease. After a successful treatment, he had a complete recovery of the psychiatry and cognitive functions. The present study also highlights the clinical features of possible ischaemic stroke resulting from

insidious cryptococcal meningitis. Headache and raised intracranial pressure were notable in these patients. This was consistent with the findings of similar studies where multiple neurological features are associated with cerebral infarct secondary to cryptococcal meningitis. [4,12]. In cryptococcal disease, cranial neuropathies, which affect cranial nerves II to VII are common, and these could lead to elevated intra cranial pressure being manifested papilledema and inflammation [4]. Other clinical factors associated with the risk of stroke in this study included vomiting, hypertension, urinary tract infection (UTI), older age and diabetes, though they were not statistical significant. There was an inverse association between Body Mass Index (BMI) and stroke types in patients with diabetes [32]. Occurrence of diabetes in this study could have been a contributory factor for the increased risk of stroke. Mortality among the CrAg positive cases was about 20% and none occurred in the control group. This is not unexpected, as patients with cryptococcal meningitis and infarcts are known to have mortality and significant neurological deficits.

Limitation of the study

Our sample size was relatively small in this pilot study and this could have affected the statistical inferences and conclusions generated by this study. It may also be difficult to generalize our finding on the Nigerian populace at large. Also, we were unable to perform cerebrospinal fluid CrAg tests to evaluate CNS involvement among the CrAg positive patients. This is important to differentiate asymptomatic cryptococcosis from meningoencepahlitis. However, this is one of the largest study on CrAg prevalence in the region.

Conclusion

Cryptococcal antigenaemia appears to be more common in stroke patients compared to controls. Infectious causes of stroke are underdiagnosed but are important to take into consideration in patients with no apparent vascular risk factor. Cryptococcal Infectious causes should be considered in the differential and workup of stroke in certain patient populations, especially in sub-Saharan Africa. In patients with confirmed infection, appropriate treatments should be initiated to minimize adverse stroke-related outcomes. Adequately powered studies are required to explore this observation.

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Contribution of the authors

SAF and MOO designed the study and planned the analysis. SAF, OBM and JN performed literature search, writing the introduction and the laboratory analysis. SAF, FB and OBM did interpretation of the data and draft manuscript. RA, SD, MF and MOO are SIREN investigators who contribute to the baseline data. All authors read the draft manuscript and provided feedback that resulted in the final manuscript.

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