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Seroprevalence of *Cryptococcus Neoformans* in Human Immunodeficiency Virus Positive Patients attending Aminu Kano Teaching Hospital Kano, Nigeria

Isa Muhammad Daneji Mulenga [1*], Abdulkadir Magaji Magashi Siziya [2] Yusuf Mohammed [1], Hamisu Umar Takalmawa [1], Almuktar Yahuza Adamu [1], Alhassan Shariff Abdullahi [1], Muhammad Ibrahim Getso [1], Fatima Muhammad Daneji [1], Mustapha Ahmed Yusuf [1]

[1] *Department of Medical Microbiology and Parasitology, Faculty of Clinical Sciences, College of Health Sciences. Bayero University Kano*
[2] *Department of Microbiology, Faculty of Life Sciences, Bayero University Kano*

* Corresponding author. +234 8032881988 *Email addresses*: ismodan@yahoo.co.uk;

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Abstract

The HIV pandemic has greatly contributed to the emergence of opportunistic fungal infections. Over the last two decades *Cryptococcus neoformans* infection is estimated to cause more than 500,000 deaths annually among HIV positive subjects in sub-Saharan Africa. However, in this environment scant attention has been paid to the screening of this infection. This study was therefore designed to determine the prevalence of *Cryptococcus neoformans* in the serum of HIV positive subjects attending HIV treatment centre in Aminu Kano Teaching Hospital, determine the relationship between the immune status with seroprevalence of the infection and to determine the risk factors associated with the infection. Blood was collected by venepuncture and CD4 count was determined using Partec flow cytometer. One hundred and fifty HIV positive subjects on Highly Active Antiretroviral Therapy with a CD4 count ≤ 200 cells/µL were recruited for the study. All subjects were grouped based on their CD4 counts into: 200-150, 149-101, 100-50 and <50 cells/µL. The test for *Cryptococcus neoformans* was performed using cryptococcal antigen (CRAG) lateral flow assay detection kits. Of 150 HIV positive subjects, 6(4.0%) were positive for serum CRAG. Positive subjects with CD4 count ≤50 cells/µL had the highest prevalence of serum CRAG. Moreover, high seroprevalence was observed in subjects that had contact with bird droppings. This study showed a prevalence of 4.0% serum CRAG among HIV positive subjects that are on HAART. It is therefore recommended to implement CRAG screening strategy targeting HIV positive subjects with lower CD4 counts.

Keywords: *Cryptococcus neoformans*, HIV, CRAG, CD4 count.

1. INTRODUCTION

The HIV pandemic has greatly contributed to the emergence of opportunistic fungal pathogens and increased incidence of infections over the last two decades. Cryptococcosis is an opportunistic mycosis, caused by an encapsulated yeast *Cryptococcus* species complex (*Cryptococcus neoformans* and *Cryptococcus gattii)* (Lin et al., 2006 and Dzoyem et al., 2012).

Cryptococcus neoformans is the most important pathogen responsible for most clinical cases among the *Cryptococcus* species complex. It has a global distribution and accounts for more than 90% of all cases of Cryptococcosis (Litvintseva et al., 2005, Eileen and John 2016).

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Recent estimates suggest that there are about 1 million new cases of cryptococcosis and at least 500,000 deaths annually worldwide due to HIV-associated cryptococcosis (Oyella et al., 2012). The vast majority of cases occur among patients living in sub-Saharan Africa because it causes more deaths than tuberculosis (Park et al., 2009). Sub-Saharan Africa carries the greatest burden of the world's Acquired Immune Deficiency Syndrome (AIDS) epidemic, with more than 60% of the world's HIV-infected population (approximately 25 million individuals) residing there (UNAIDS, 2006). Exposure to *Cryptococcus neoformans* is thought to be universal. The organism is inhaled from the environment, and genotypic evidence suggests acquisition can occur many years before the development of clinical cryptococcosis in the context of immunosuppression (Velagapudi et al., 2009).

Cryptococcal antigenemia (presence of cryptococcal capsular polysaccharide antigen (CRAG) in blood), can precede onset of cryptococcal meningitis (CM) by weeks to months (French et al., 2002). The World Health Organization noted that the main reason for the unacceptably high mortality is a delay in presentation when meningitis is advanced and treatment is less effective. If diagnosis could be made earlier, the burden of cryptococcal disease may be drastically reduced. It can be seen that cryptococal infection is an important public health problem and causes a large burden of disease among people living with HIV/AIDS (WHO, 2011).

The prevalence of cryptococcosis in Africa and most especially in Nigeria is not well documented due to poor recording of statistical data and low research level. In immunocompromised patients, cryptococcosis can be severe and rapidly progressive; requiring prolonged systemic antifungal therapy (Saag et al., 2000). The infection is the most common life-threatening disease in HIV patients, and extra- pulmonary cryptococcosis is an AIDS- defining illness. The clinical presentation includes disseminated meningo-encephalitis, which is rapidly fatal in the absence of therapy (Mwaba et al., 2001). This presents with high morbidity in HIV/AIDS patients in sub-Saharan Africa. In this region, prospective studies on cryptococcal pulmonary infections are needed so that appropriate therapy can be initiated, thus reducing the incidence of opportunistic infections. (Junaid et al., 2008).

Few data on cryptococcosis in HIV's patient are available in Nigeria, , hence the need of for a study like this to find out its prevalence in HIV patients. Cryptococcosis has emerged as defining feature for HIV/AIDS infection (Junaid et al., 2008). Patients with AIDS associated cryptococcal infections now account for 80 – 90% of all patients with cryptococcosis (Wallace et al., 2008). A recent study in Africa reported the prevalence of cryptococcal antigenemia in HIV patient cohorts with CD4 counts below 100 cells/μL at ranges from 2 to 13% (French et al., 2002).The seroprevalance of *Cryptococcus neoformans* on population groups considered to be at risk of acquiring an infection will therefore be investigated.

In recent times, the incidence of cryptococcal meningitis in patients infected with HIV has increased worldwide mainly because of the increased awareness on the part of both the physicians and clinical microbiologists (Ashiru et al., 2005). It generally occurs when the CD4+ T cell count is less than 100cells/ μL and presents in descending order as cryptococcal meningitis, pulmonary cryptococcosis and cryptococcosis of the skin (Salami et al., 2009).

A presumptive diagnosis of CM can be made on the basis of a positive cryptococcal antigen (CrAg) using a latex agglutination or an enzyme immumo assay kit. Cryptococcal antigene testing on blood and cerebrospinal fluid (CSF) is a highly reliable and rapid diagnostic technique with excellent sensitivity and spec ificity (Antinori et al., 2005). In one study, of the 59 patients with cryptococcal infection in which 17 (28.8%) would not have been diagnosed on the day of consultation without the agglutination test performed on serum (Micol et al., 2007). For this reason, prophylaxis of antifungals should be given to HIV positive patient with lower CD4 counts 400 cell/μL and a positive serum CRAG to prevent them from having any form of cryptococcosis.

2. METHODS

Data Collection

A structured questionnaire was used for data collection. The questionnaire included demographic and clinical data including, age, gender, clinical features, and CD4 cell counts of study participants.

Specimen Collection / Storage

Five millilitres of whole blood samples was collected from each participant by venipuncture through the help of a volunteered experienced phlebotomist into a pre-labeled EDTA and plain tubes, two millilitres of withdrawn blood was immediately used for CD4 cell count measurement by flow cytometry (S.L Green ,Partec, Germany) according to the manufacturer's instructions (Fig. 1).

Figure 1: Partec Cyflow machine used for measurement of CD4 cell count

Blood samples from all study participants was spun at 3,000 rpm for 10 min and the serum samples obtained. Specimens were transferred into pre-labelled cryovials (Fig. 2) and frozen at -20°C, Specimens (serum) in transit were maintained at 8°C (Dzoyem et al., 2012).

Principle of Lateral Flow Assay

The CrAg Lateral Flow Assay is a dipstick sandwich immunochromatographic assay. Specimens and specimen diluent are added into an appropriate reservoir, such as a test tube, and the lateral flow device is placed into the reservoir. The test uses specimen wicking to capture gold-conjugated, anti-

CrAg monoclonal antibodies and gold-conjugated control antibodies deposited on the test membrane. If CrAg is present in the specimen, then it binds to the gold-conjugated, anti-CrAg antibodies.

Figure 2: Subjects sera used

The gold-labeled antibody-antigen complex continues to wick up the membrane where it will interact with the test line, which has immobilized anti-CrAg monoclonal antibodies. The gold-labeled antibody-antigen complex forms a sandwich at the test line causing a visible line to form. With proper flow and reagent reactivity, the wicking of any specimen, positive or negative, will cause the gold-conjugated control antibody to move to the control line. Immobilized antibodies at the control line will bind to the gold-conjugated control antibody and form a visible control line. Positive test results create two lines (test and control). Negative test results form only one line (control). If a control line fails to develop then the test is not valid (Fig. 3).

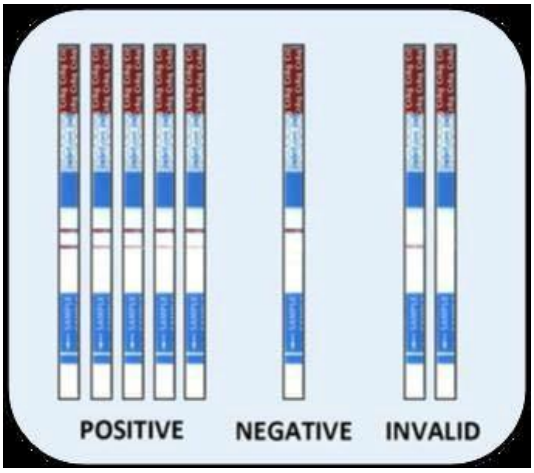

Figure 3: Interpretation of lateral flow assay test

Procedure for Lateral Flow Assay

Qualitative Procedure

One drop of LF Specimen Diluent (GLF025) was added to an appropriate container (micro-centrifuge tube). Then 40 µL of specimen (serum) was also added to the container. The white end of a Cryptococcal Antigen Lateral Flow Test Strip (LFCR50) was submerged into the specimen.This lasted for 10 minutes, and then the results were read and recorded according to the manufacturer's instructions.

Semi-Quantitative Titration Procedure

Ten micro-centrifuge tubes were placed in an appropriate rack and labeled 1-10 (1:5 through 1:2560). Additional dilutions were necessary if the specimen is positive at 1:2560. Four (4) drops of LF Specimen Diluent (GLF025) was added to tube number 1. Then 2 drops of LF Titration diluent (EI0010) was also added to each of the tubes labeled 2-10. Forty microlitre (40 µL) of specimen (serum) was added to tube number 1 and mixed well. Eighty microlitre (80µL) of specimen was transferred from tube number 1 to tube number 2 and mixed well. This dilution procedure was continued through tube number 10. The white end of a Cryptococcal Antigen Lateral Flow Test Strip was submerged into the specimen in each of the 10 tubes. They were allowed to wait for 10 minutes. Finally, the results were read and recorded according to the manufacturer's instruction (Immune mycologics, Inc, technology place Norman USA).

Data Analysis

Statistical analyses were carried out using SPSS version 20. Chi square (X^2) test was used to compare baseline demographic and clinical characteristics between serum CRAG-positive and CRAG-negative subjects. A p value of less than 0.05 was used as indicative of statistical significance.

Area Descriptions

The study was carried out at Prof Sadiq Wali treatment centre, which is the retroviral treatment clinic located in Aminu Kano Teaching Hospital. This center is supported by the Institute of Human Virology

of Nigeria (IHVN) – PEPFAR project with a grant from the Centre for Disease Control and Prevention (CDC). It has both clinical and laboratory facilities for treatment and follow up of HIV infected patients.

Study Design

A cross sectional design was used for this study.

Study Population

The study population consisted of all HIV positive subjects attending Professor Sadiq Suleiman Wali centre in Aminu Kano Teaching Hospital between January to December, 2015

Inclusion Criteria

The study included all HIV positive subjects with CD4 count of ≤ 200 cells/μL and on highly active antiretroviral therapy (HAART).

Exclusion Criteria

This study excluded all HIV subjects with CD4 count of > 200 cells /μL.

Sample Size Determination

The sample size was determined using the formula:

$$nn = \frac{(ZZ^2 \times PP \times QQ)}{dd^2}$$

Where:

n = minimal sample size
Z = confidence limit = 95% (1.96)
P = prevalence rate observed from previous study 9.86% = 0.0986 (Dzoyem et al., 2012)
q = 1 – p
d = tolerable error 5% = 0.05

$$nn = \frac{(1.96^2 \times 0.0986 \times 0.901)}{0.05^2} = 137$$

The minimum sample size is 137 rounded to 150

Ethical Clearance

Ethical clearance was sought and granted from the AKTH research ethics committee. The study aim was explained clearly to the participants and informed consent was obtained before proceeding to the study.

3. RESULTS

The overall prevalence of positive serum cryptococcal antigen (CRAG) by qualitative test was 4.0% (Table 1 and Fig. 4).

The age of the participants ranged between 16-58 years. The mean age of the subjects was 37.03 ± 9.72. The age of the subjects was categorized into four groups ranging from 10-19, 20-29, 30-39, 40-49 and 50-59 (Table 1).

Table 1: Seroprevalence of CRAG among HIV Subjects in Kano State.

	Result		
Age (Years)	**Total Number**	**Total Positive N (%)**	**P. Value 0.435**
10 – 19	3	0 (0)	
20 – 29	33	1 (0.7)	
30 – 39	52	1 (0.7)	
40 – 49	44	2 (1.3)	
50 – 59	18	2 (1.3)	
Total	**150**	**6 (4.0)**	

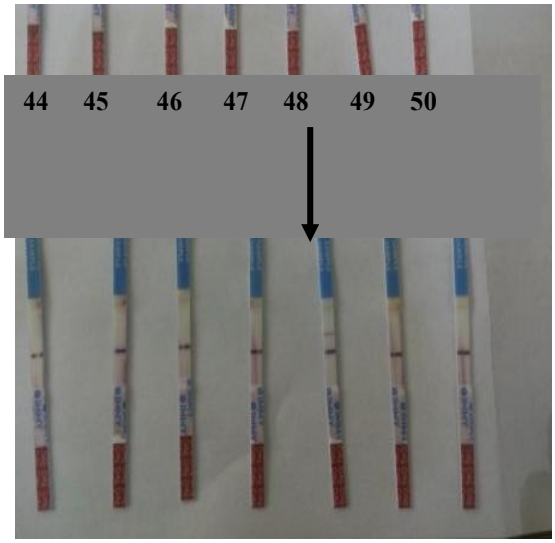

Figure 4: Serum CRAG qualitative test. Sample no 48 shows positive test result and the others are negative

Majority of the subjects 34.7% were in the age group of 30-39 years, 29.3% were in the age group of 40 -49 years, 22.0% were in the age group of 20 – 29 years, 12.0% were in the age group of 50 – 59 years and 2.0% were in the age group of 10 – 19 years (Table 2).

Positive Serum CRAG also showed highest prevalence of 11.1% among age groups of 50-59 years, followed by a prevalence of 4.5 % in the 40-49 age group. A prevalence of 3% was seen in the 20-29 age group, 1.9% was also recorded in 30-39 age group and none was recorded in 10-19 age group (Table 2).

The sex distribution of the 150 patients screened was 68% males and 32% females. (Table 2).Among the males screened, 2.9% had a positive serum CRAG, while 6.2% of the females screened had a positive serum CRAG result (Table 2). The marital status of the subjects was categorized into four groups: single, married, widowed and divorced. Married subjects constituted the highest number 64% followed by widowed group of 12.7%, 12% were single, while 11.3% were divorced. Among the married subjects, 6 were positive of serum CRAG which constitutes 6.2% (Table 2).

In terms of educational background, 34.7% had secondary school education, 25.3% had tertiary education, 24% had Qur'anic education, 14.7% had primary school education, while 1.3% had no formal education (Table 2). The seroprevalence of *Cryptococcus neoformans* in terms of level of education was found to be highest 9.1% in those with primary education. Those who had tertiary level of education were 5.3%, and those with secondary level of education constitutes 3.8% of those tested for Serum CRAG (Table 2). Most of the subjects 82% reside in the urban areas while 18% are from rural areas. Those from the rural areas had the highest CRAG seroprevalence of 14.8% (Table 2).

Table 2: Seroprevalence of CRAG according to demographic characteristics of the subjects.

Characteristics	**Result**			**P. Value**
	Positive N (%)	**Negative N (%)**	**Total N (%)**	
Age (Years)				**0.435**
10 – 19	0 (0)	3(100.0)	3 (2.0)	
20 – 29	1 (3.0)	32 (97.0)	33 (22.0)	
30 – 39	1 (1.9)	51(98.1)	52(34.7)	
40 – 49	2 (4.5)	42 (95.5)	44 (29.3)	
50 – 59	2 (11.1)	16(88.9)	18(12.0)	
Total	6 (4.0)	144 (96.0)	150 (100)	
Sex				**0.395**
Male	3 (2.9)	99 (97.1)	102 (68.0)	
Female	3 (6.2)	45 (93.8)	48 (32.0)	

Total	6 (4.0)	144 (96.0)	150 (100)	
Marital Status				**0.246**
Single	0 (0)	18 (100.0)	18 (12.0)	
Married	6 (6.2)	90 (93.8)	96 (64.0)	
Widowed	0 (0)	19 (100.0)	19 (12.7)	
Divorced	0 (0)	17 (100.0)	17 (11.3)	
Total	6 (4.0)	144 (96.0)	150 (100)	
Educational Status				**0.388**
Primary	2 (9.1)	20 (90.9)	22 (14.7)	
Secondary	2 (3.8)	50 (96.2)	52 (34.7)	
Tertiary	2 (5.3)	36 (94.7)	38 (25.3)	
Qur'anic	0 (0)	36 (100.0)	36 (24.0)	
None	0 (0)	2 (100.0)	2 (1.3)	
Total	6 (4.0)	144 (96.0)	150 (100)	
Residence				**0.010**
Urban	2 (1.6)	121 (98.4)	123 (82.0)	
Rural	4 (14.8)	23 (85.2)	27 (18.0)	
Total	6 (4.0)	144 (96.0)	150 (100)	

There is statistically significant difference between those residing in rural and urban areas (P = 0.010). The study subjects were categorised based on the duration of HIV infection in years, 46.7% have duration of 1-5 years, 32.7% have duration of 6-10 years HIV infection, 17.3% have duration of < 1year and 3.3% have duration of > 11years (Table 3). The highest serum CRAG prevalence of 20% was recorded in those with HIV infection of more than 11years, 6.1%, 2.9% were recorded in 6-10years, 1-5years respectively, and < 1year had none (Table 3).
The highest seroprevalance 13.0% was observed in those that had contact with bird droppings (Table 3).

Table 3: Seroprevalence of CRAG in relation to the duration of HIV and the possible sources of the infection.

Duration of HIV (Years)	**Result Positive N (%)**	**Negative N (%)**	**Total N (%)**	**P. Value 0.162**
< 1	0 (0)	26 (100)	26 (17.3)	
1 – 5	2 (2.9)	68 (97.1)	70 (46.7)	
6 – 10	3 (6.1)	46 (93.9)	49 (32.7)	
> 11.	1 (20.0)	4 (80.0)	5 (3.3)	
Total	6 (4.0)	144 (96.0)	150 (100)	
Contact With Bird Droppings				**0.047**
Yes	3 (13.0)	20(87.0)	23 (15.3)	
No	3 (2.4)	124 (97.6)	127 (84.7)	
Total	6 (4.0)	144 (96.0)	150 (100)	
Blood Transfusion				**0.311**
Yes	2 (7.1)	26 (92.9)	28 (18.7)	
No	4 (3.3)	118 (96.7)	122 (81.3)	
Total	6 (4.0)	144 (96.0)	150 (100)	

There is strong relationship ($p < 0.05$) between positive serum CRAG and contact with bird droppings in this study.

Moreover 18.7 % had history of blood transfusion while 81.3% have never been transfused with blood. There is no significant statistical association between cryptococcal infection and blood transfusion as $p > 0.05$(Table 3).

Subjects were categorized according to the CD4 count ranges, 28.7% had a CD4 count range of 200 – 150 cells/µL, 26.7% had a CD4 count of 149 – 101 cells/µL, 23.9% had a CD4 count range of 100 – 50 cells/µL and 20.7% had a CD4 count range of <50 cells/µL. Subjects with CD4 cell counts > 50 cells/µL had the highest prevalence of serum CRAG of 9.7% , this was followed with 2.8% in CD4

count range of 100 – 50 cells/µL, then 2.5% in CD4 count range of 149 – 101 cells/µL and 2.3% in CD4 count range of 200 – 150 cells/µL (Table 4).

Table 4: Seroprevalence of CRAG in relation to The CD4 count of the subjects.

	Result			
CD_4 Count (Cells/µL)	**Positive N (%)**	**Negative N (%)**	**Total N (%)**	**P. Value 0.491**
200 – 150	1 (2.3)	42 (97.7)	43 (28.7)	
149 – 101	1 (2.5)	39 (97.5)	40 (26.7)	
100 – 50	1 (2.8)	35 (97.2)	36 (24.0)	
< 50.	3 (9.7)	28 (90.3)	31 (20.7)	
Total	**6 (4.0)**	**144 (96.0)**	**150 (100)**	

The packed cell volume (PCV) of the subjects was categorized into: 36 - 50%, 30 - 35.9% and 21 – 29.9% ranges. Forty two percent had a PCV range of 30 - 35.9%, 40% have a PCV range of 36 - 50% and 18% have a PCV range of 21 - 29.9% .The highest prevalence of serum CRAG of 6.3% was found in PCV range of 30 - 35.9%. (Table 5)

Table 5: Seroprevalence of CRAG in Relation to the PCV of the Subjects.

	Result			
Packed Cell Volume (%)	**Positive N (%)**	**Negative N (%)**	**Total N (%)**	**P. Value 0.561**
36 – 50	2 (3.3)	58 (96.7)	60 (40.0)	
30 – 35.9	4 (6.3)	59 (93.7)	63 (42.0)	
21 – 29.	0 (0)	27 (100)	27 (18.0)	
Total	**6 (4.0)**	**144 (96.0)**	**150 (100)**	

For the semi-quantitative titration result, 1 sample was positive upto a titre of 1:1280, one sample had a titre of 1:10, two samples had a titre upto 1:5 and the other 2 samples were positive on a neat dilution i.e. upto a titre of 1:1 (Fig. 5).

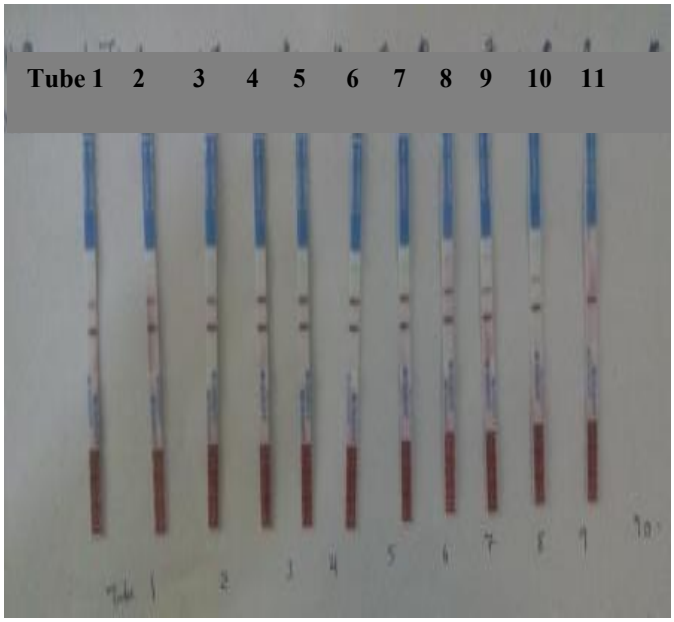

Figure 5: Figure showing semiquantitative titration method.

4. DISCUSSION

This research documented an overall prevalence of 4.0% of serum CRAG positive for *Cryptococcus neoformans* in HIV subjects with CD4 count ≤ 200 cells/µL in Kano State. The prevelance in this study is in agreement with another study carried out in Anambra, Nigeria which documented 4.2% (Chukwuanukwu et al., 2013b.). Similarly another study in Tanzania revealed a prevalence of 4.4% (Wajanga et al., 2011).

In a similar study, Liechty et al., (2007) reported a prevalence of 5.8% serum CRAG titre ≥1:2. However the study was done to relate serum CRAG and early mortality in rural Uganda among individuals with advanced HIV disease. The prevalence documented in this study is in concordance with another study reported in London with a prevalence of 5% (Patel et al., 2013). However, the study was done on newly diagnosed HIV patients with CD4 counts of ≤100 cell/µL, whereas in this study patients recruited where on HAART and not newly diagnosed, who have a CD4 count of ≤200 cells/µL.

Moreover, a similar study reported a higher prevalence of 13.1% among HIV positive pregnant women attending a prevention of mother to child transmission (PMTCT) clinic in South-East Nigeria (Chukwuanukwu et al., 2013a.). A prevalence of 12.7% was reported in Benin City, Nigeria by Osazuwa et al., (2012) in a study conducted on anti-retroviral naïve AIDS patients. This might be a probable reason why a high prevalence was recorded in that study. High prevalence of 9.91% was reported in the Benin City, Nigeria in patients who are on anti-retroviral drugs with different ranges of CD4 cell counts (Egbe et al., 2015). Another high prevalence of 9.7% was also documented in Benin (Aluyi et al., 2010). However, in that study, sputum samples of AIDS patients were used to culture *C. neoformans* and not serum samples. Similarly, low prevalence of 1.4% was reported of *Cryptococcus neoformans* in a survey of bacterial and fungal opportunistic infections among HIV clients in Kano metropolis (Usman and Uba 2011). However, the sample used in the study was sputum as against to serum that was used in this study. The use of serum in the diagnosis of cryptococcosis has improved significantly with the development of serologic tests for the cryptococcal polysaccharide capsular antigen (CrAg), which is shed during infection (Eileen and John 2016). In parts of Africa, a high prevalence of 19% was recorded in Kampala, Uganda among patients with CD4 count of ≤100 cells/µL i.e among severely immunosuppressed HIV infected patients (Oyella et al., 2012). Similarly, a prevalence of 8.2% in Uganda was documented among HIV-infected persons with a CD4 cell count ≤100cells/µL who start HIV therapy in resource-limited settings (Meya et al., 2010). A prevalence of 7% was also documented in Cape town, South Africa as a result of screening for cryptococcal antigenemia in patients accessing ARV treatment program (Jarvis et al., 2009). In Ghana, a low seroprevalance of 2% of cryptococcal antigenemia in patients with advanced HIV infection was documented (Mamoojee et al., 2011), however only ninety-two serum samples from adults with CD4 counts <100cells/µL was used in that study.

Moreover, other higher prevalences in other parts of the world include 18% in a study that was conducted among HIV-infected patients in 2004 in Cambodia, Thailand (Micol et al., 2007). A prevalence of 13.1% was also documented among HIV infected patients hospitalized with pneumonia in two rural provinces in Thailand (Harris et al., 2012). A prevalence of 9.2% was also documented in another study for the early diagnosis of cryptococcosis in HIV-infected patients with different ranges of CD4 cell counts (Pongsai et al., 2010). Similarly, a prevalence of 2.5% of *Cryptococcus neoformans* was documented in Isfahan, Iran (Soltani et al., 2013), their study was on isolation of *Cryptococcus neoformans* from pigeon droppings and not on serum samples.

Age did not play a significant role in serum CRAG positivity in the patients used in this study. Subjects within the age group of 50-59 years recorded the highest prevalence of 11.1% of serum CRAG positivity. The most probable reason could be due to weakness of immune system as patients are ageing, thereby making them highly vulnerable to infection by opportunistic mycoses (Aluyi et al., 2010).

On the basis of sex, females had more seropositivity for serum CRAG. Compared to their male counterparts (4Females: 2males). Similar case was reported by Aluyi et al., (2010) where he reported the occurrence of opportunistic pulmonary mycoses to be higher in females 86 (44.1%) than in males 80 (41. 0%). But there was no statistically significant difference (P= 0.385) in the prevalence of serum CRAG in HIV subjects attending AKTH clinic in Kano.

Moreover, this study revealed that subjects from rural areas had the highest prevalence of serum CRAG of 14.8%. This was statistically significant (p= 0.010) there is a relationship between those living in rural areas and seropositivity to CRAG. The reason may be due to the fact that people living in rural areas have poor health seeking behavior and also they need to travel a far distance before reaching tertiary health centers which are located in urban areas of Kano.

Avian droppings have been reported as a potential environmental source of human pathogenic yeast. Pigeon droppings which are abundant in public areas, could especially be a potential carrier in the spread of pathogenic yeast into the environment subsequently humans (Chee et al., 2005). In this research a prevalence of 13.0% was observed in those that had contact with bird droppings. Moreover, this also showed a statistically significant relationship between contact with bird droppings. A similar prevalence of 13% was reported by Irokanulo et al., (1997) in Jos. However, his study was on isolation of *Cryptococcus neoformans* from droppings of captive birds in Jos wildlife park and Zoo. Another high prevalence of 14.4% was reported in Awka, Nigeria (Mbata et al., 2006), the study was on isolation of *Cryptococcus neoformans* from bats (*Molossus major*) droppings. A similar study by Nweze et al., (2015) carried out in Southeastern Nigeria reported a prevalence of 22.0%, but the research was on isolation of *Cryptococcus neoformans* from environmental samples of pigeon droppings and not on contact with bird droppings. In Iran, a study reported a low prevalence of 2.5% of *Cryptococcus neoformans* isolated from pigeon droppings (Soltani et al., 2013).

The distribution of cryptococcal antigenemia was highly varied with CD4 cell count levels. Patients with CD4 cell counts of ≤50 cell/µL had the highest prevalence of serum CRAG reported in this study. In a recent study Osazuwa et al., (2012) reported that out of 150 ART naïve AIDS patients with CD4 counts ≤ 200 cells/µL, in which 19 (12.7%) were positive for serum CRAG. Antiretroviral therapy (ART) naïve AIDs patients with CD4 count ≤ 50 cells/µL had the highest prevalence of serum CRAG. Several studies evaluating the prevalence of serum cryptococcal antigememia in AIDS patients have reported a consistently higher prevalence of serum CRAG in patients with lower CD4 cell counts; 14.3% in CD4 count < 50 cells/µL (Micol et al., 2007), 12.9% in CD4 count < 100 cells/µL (Pongsai et al., 2010), 28.2% in CD4 count < 50 cells/µL (Osazuwa et al., 2012). This trend was also observed in this study with a 9.7% in CD4 count < 50 cells/µL.

5. CONCLUSION

This study showed a prevalence of 4.0% among subjects who are on HAART attending AKTH clinic in Kano State. This is more common among individuals with CD4 count ≤ 50 cells/µL as such serum CRAG screening has an important role for the early diagnosis of cryptococcal infection in such type of subjects with low CD4 cell counts.

Furthermore this study showed that living in rural area could be an important factor in acquiring the cryptococcal infection.

Moreover this study also showed a significant association of serum CRAG with contact with bird droppings in acquiring the cryptococcal infection. As such the education of residents especially those that are HIV positive on the epidemiological relevance of bird droppings in the environment is needed.

6. RECOMMENDATIONS

Given the results of this study, screening for cryptococcal antigenemia should be carried out routinely on all HIV subjects irrespective of whether patient is on HAART or not so as to minimize the morbidity and mortality of cryptococcosis.

Routine universal screening should be performed on immunosuppressed or HIV positive subjects at high risk of cryptococcal infection.

Further studies should be carried out on HIV subjects that are not on HAART (ARV-naive) in this State and other parts of the country.

Moreover, there is need for further studies on the isolation of *Cryptococcus neoformans* from bird droppings in this part of the country.

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