Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.3, No.18, 2013

Cryptococcus neoformans Antigenemia in HIV Positive Pregnant Women Attending a PMTCT Clinic in South-East Nigeria

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Abstract
Cryptoccocus neoformans infection is life threatening especially when associated with HIV disease. Unfortunately, in our environment, scant attention has been paid to screening for this fungal disease despite reports of relatively high co-infection rates with HIV in other countries facing similar HIV/AIDS burden. Among people living with HIV/AIDS, there is a significant population of pregnant women. In pregnancy itself, there is immunosuppression, thus combining with the immunosuppression seen in HIV disease. This study was therefore designed to determine the prevalence of *cryptoccocus neoformans* infection, among HIV seropositive pregnant women attending a prevention of mother to child transmission (PMTCT) of HIV treatment centre. Two hundred and eighty (280) women were recruited for the study. One hundred and sixty (160) of the subjects were HIV seropositive (test group) while 120 HIV seronegative pregnant women served as controls. The test group was sub-divided into three groups based on their CD4 counts: <200, 200-300 and >300 cells l. Blood was collected by venipuncture and HIV status was determined by current national serial algorithm using Determine and Stat Pak test kits. The test for *Cryptoccocus neoformans* was performed using latex cryptococcal antigen (CRAG) detection kits.CD4 counts was determined using the Partec cyflow analyzer. A prevalence rate of 13.1% was observed among HIV seropositive subjects, while none (0%) of the control group tested positive. Cryptococcal antigenemia correlated with decreasing CD4 counts with most of the positive subjects having CD4 counts below 200 cells/ l. It is recommended that targeted cryptococcal screening be made a part of baseline tests in HIV positive pregnant women with low CD4 + T cell counts. This will reduce preventable deaths and improve obstetric outcome in this vulnerable group.
Keywords: *Cryptoccocus neoformans,* HIV/AIDS, Pregnancy

Introduction

Cryptococcosis is responsible for an estimated one million cases of meningitis per year, predominantly in HIV infected subjects (Alanio *et al,* 2011), and in sub-Saharan Africa, has been associated with 17% of all deaths among HIV-infected patients (French *et al,* 2002). There are two pathogenic variants: *Cryptococcus neoformans* var neoformans, which causes majority of the disease in persons with AIDS (Mitchell and Perfect, 1995), and *Cryptococcus neoformans* var gatti which rarely causes the disease in AIDS (Litvintseva *et al*, 2005). Cryptococcosis is acquired via inhalation of aerosolized particles from the environment (Saha *et al*, 2007). After inhalation, it is believed to lie dormant for many years. Reactivation, which occurs permanently among immunosuppressed individuals such as persons with HIV/AIDS, leads to infection, the common of which is meningitis (Roy and Chiller, 2011). AIDS associated cryptococcal meningitis (CM), caused by *Cryptococcus neoformans,* is a severe opportunistic infection with a high mortality, even in developed countries (Bicanic and Harrison, 2005). In Africa, CM is responsible for 13% to 42% of all deaths among HIV-infected people (Mwaba *et al*, 2001). CM is the AIDS-defining illness in 25% to 30% and 64% to 91% of cases in South-East Asia (Iyer and Banker, 2002) and sub-Saharan Africa (Schaars *et al,* 2006) respectively. *C. neoformans* is hypothesized to cause a sub clinical pulmonary infection which can evolve to a quiescent latent state with the potential for later reactivation in the context of acquired immunosuppression (Saha *et al*, 2007). Cryptococcal disease cannot be passed from person to person or from animal to person. A person with cryptococcal disease is not contagious (CDC, 2011).The fungus can affect a person's lungs (Pneumonia) or the Central nervous system (Meningitis). *C. neoformans* is a common cause of meningitis in HIV/AIDS (Dismukes, 1998). According to the CDC, cryptococcal meningitis is a leading cause of death among persons living with HIV/AIDS in sub-Saharan Africa. With the advent of HIV/AIDS, the incidence of cryptococcosis is increasing and now represents a major threat of fungal infections in HIV patients. The HIV epidemic has facilitated an increase in the incidence of this infection, with HIV co-infection implicated in 80% of cases of cryptococcosis worldwide (Chayakulkeeree and Perfect, 2006). Furthermore, with the changes in the demography of Human Immunodeficiency Virus (HIV) infection, women and children are becoming the fastest growing groups of newly infected patients with Cryptococcus neoformans (Lipman *et al*, 2003). Among people living with HIV/AIDS, there is a significant population of

pregnant women. Pregnancy represents a vulnerable time for both mother and fetus, and is considered a period of relative immune suppression (Costa *et al*, 2009). In the pregnant state, the mother's immunological status is downregulated in part to prevent fetal rejection due to paternally-derived histocompatibility antigens (Catanzara, 1984). This immunosuppression is likely to be more severe in women infected with HIV; thus, they may be more susceptible to opportunistic infections like Cryptococcosis and post-surgical complications (Kwalombota, 2002). There is still no clear evidence that pregnancy is a predisposing factor for cryptococcosis, however, alteration of maternal T-cell activity, natural killer cells, polymorphonuclear leucocytes, macrophages and specific antibodies may play an important role in the development of this disease (Ely *et al*, 1988). Thus pregnancy could create a predisposition to opportunistic fungal infections due to relative immunosuppression (Ely *et al*, 1988).With increasing access to anti-retrovirals, opportunistic infections in HIV are declining. It has been reported though that only 37% of those in sub-Saharan Africa who are eligible for ART are receiving it (WHO/UNAIDS, 2010). Quite a number of the HIV infected do not present to hospital for medical care till they have progressed to AIDS. In individuals who have progressed to AIDS and/or have low CD4+ T cell counts, cryptococcosis is still a huge problem. 4.2% cryptococcus antigenemia was found among HIV positive individuals in a previous study in this environment (Chukwuanukwu *et al*, 2013). Thus, the aim of this study is to determine cryptococcal antigenemia among HIV seropositive pregnant women accessing ante-natal care in a Prevention of Mother to Child Transmission (PMTCT) of HIV treatment centre.

SUBJECTS AND METHODS

Subjects

Two hundred and eighty (280) women were recruited for the study. One hundred and sixty (160) of the subjects were HIV seropositive (test group) while 120 HIV seronegative pregnant women served as controls. The participants were all pregnant women within the age of 18 and 40 years. The test group was divided into three categories based on their CD4 count; they are, those with CD4 count <200, a category with CD4 count between 200-300 and another category with CD4 count >300 cells/ l. One hundred and three (103) of the test group were on first line and second line antiretroviral drugs. Forty-four of these had been on antiretrovirals for six months or less. The participants were registered patients of HIV PMTCT clinic in Regina-Caeli Hospital Awka, Anambra State.

Inclusion and Exclusion Criteria

This study was delimited to the following: Apparently healthy pregnant women that tested negative to HIV (control group) and pregnant women that tested positive to HIV. Non pregnant women were excluded.

Ethical Consideration

Ethical approval for this research was obtained from the Ethical committee of the Faculty of Health Sciences and Technology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus. The consent of the subjects was also obtained.

Methods

Blood was collected by venipuncture and HIV status was determined by current national serial algorithm using Determine and Stat Pak test kits. The test for *Cryptoccocus neoformans* was performed using latex cryptococcal antigen (CRAG) detection kits.CD4 counts was determined using the Partec cyflow analyzer.

Determination of HIV status

The tests were carried out according to the manufacturer's instruction.

Chembio HIV 1/2 STAT PAK^{TM} assay

Principle of the test

The chembio HIV 1/2 STAT PAK^{TM} assay employs a unique combination of a specific antibody- binding protein, which is conjugated to colloidal gold dye particles and HIV1/2 antigens which are bound to the membrane solid phase. The sample is applied through the sample well followed by the addition of running buffer. The buffer facilitates the lateral flow of the released products and promotes the binding of antibodies to the antigens. If present, the antibodies bind to the gold conjugate antibody-binding protein. In a reactive sample, the dye-conjugate immune complex migrates through nitrocellulose membrane and is captured by the antigens immobilized in the test (T) area producing a pink/purple line. In the absence of HIV antibodies, there is no pink/purple line in the test (T) area. The sample migrates along the membrane and produces a pink/purple colour on the control (C) area containing immunoglobulin antigens. This control serves to demonstrate that specimens and reagents have been applied properly and have migrated through the device.

DETERMINE ^{TM} FOR HIV -1/2 Detection

The abbot Determine^{TM} HIV ½ is in vitro test kit, visually qualitative immune assay for the detection of antibodies to HIV – 1 and HIV – 2 in human serum, plasma or whole blood.

Principle of the test

Determine HIV ½ is an immunochromatographic test for the qualitative detection of antibodies to HIV-1 and HIV – 2. Sample migrates though the conjugate pad, it reconstitutes and mixes with the selenium colloid-antigen conjugate. This mixture continues to migrate through the solid phase to the immobilized recombinant antigens and synthetic peptides in HIV – 1 or HIV – 2. If HIV ½ were present in the sample, the antibodies bind to the antigen-selenium colloid and to the antigen at the patient window side. If antibodies to HIV-1 and or HIV – 2 are absent. The antigen selenium colloid flows past the patient window and no red line are formed at the patient window site. To ensure assay validity, a procedural control bar is incorporated in the assay device.

CD4 T cell determination

Principle of CD4 measurement

Flow cytometry is a process by which cells or micropartilces in suspension are differentiated and counted accordingly to the cell size, florescence, emission, and internal structure. In the cyflow counter, the fluorescence monoclonal antibody binds to the CD4 antigen in a buffer suspension. The complex is passed through the flow cuvette in a single stream flow. The complex is excited by the solid state at a wavelength of 532nm causing the emission of light by the complex which is captured by photomultiplier tube and transmitted into digital read out device as counts.

Latex Cryptococcal Antigen Detection

The test for *Cryptoccocus neoformans* was performed using Cryptococcus antigen latex agglutination test system by IMMY.Procedure was according to the manufacturer's instruction.

Principle:

The Latex Cryptococcus Antigen Test is based upon the principle that anti-cryptococcal anti-body coated latex particles will agglutinate with specimens containing Cryptococcal capsular polysaccharide antigen. Previously, the detection of this antigen in serum was hampered by the presence of rheumatoid factor. Pretreatment of serum specimen either pronage (REF DE0010) reduces nonspecific interference and enhances the detection of capsular polysaccharide antigen

Results

The findings from this study revealed positive cryptococcal antigenemia in the test subjects who were HIV positive. A prevalence rate of 13.1% was observed among HIV seropositive subjects (21 of the 160 test subjects tested positive), see fig 1, while none (0%) of the control group tested positive.

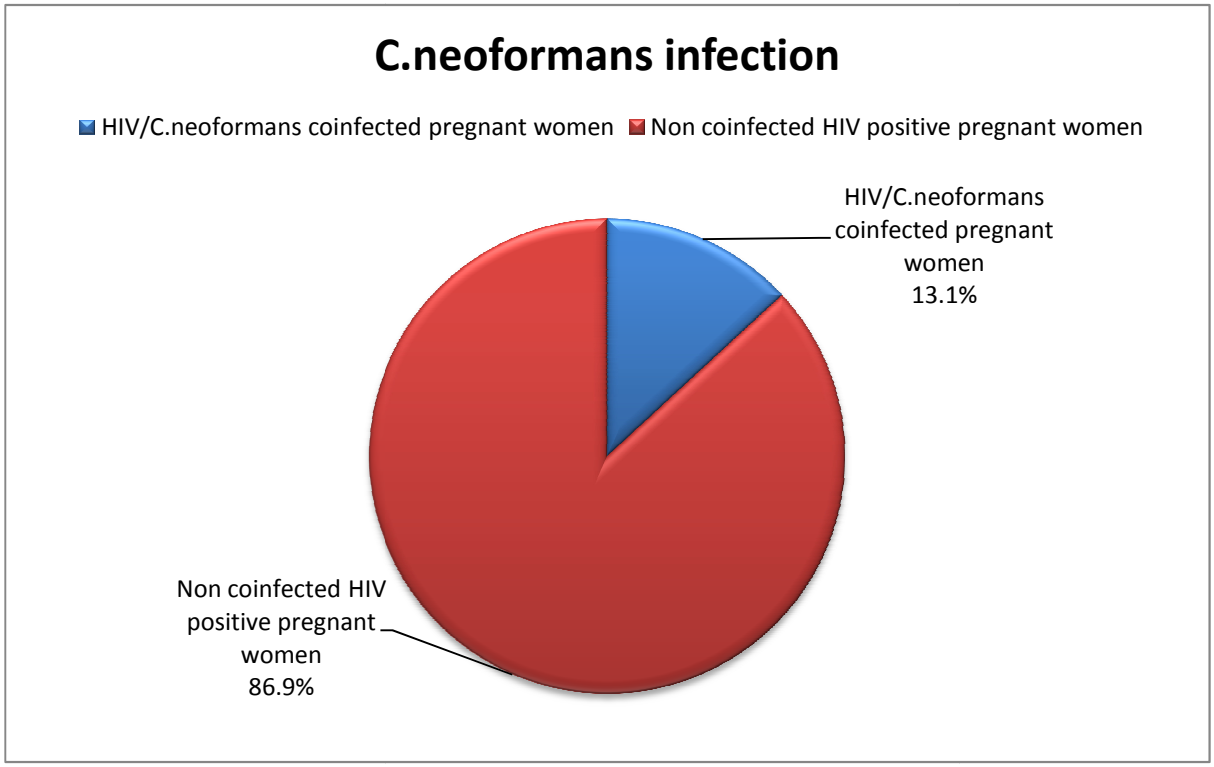

Fig 1: *Cryptococcus neoformans* status in HIV positive women attending PMTCT clinic

Of the Twenty-one (21) subjects who were coinfected with *Cryptococcus neoformans*, eleven (11) had CD4 counts below 200 cells l (52.4%), while nine (9) had CD4 counts between 200-300 cells/ l, (42.8%). Only one subject was positive when the CD4 count was above 300 cells/ l (CD4 T cell count of 354 cells/ l). None of the other subjects who had CD4 T cell count in the normal range tested positive for *C. neoformans*. The subject who had the highest CD4 count of 354 and was coinfected, had commenced anti-retroviral therapy seven (7) weeks prior to inclusion in the study. There was a significant decrease in the mean CD4 count levels of HIV sero-positive subjects co-infected with *Cryptococcus neoformans* when compared with HIV sero-positive subjects not infected,($P<0.05$), thus, cryptococcal antigenemia correlated with decreasing CD4 counts.

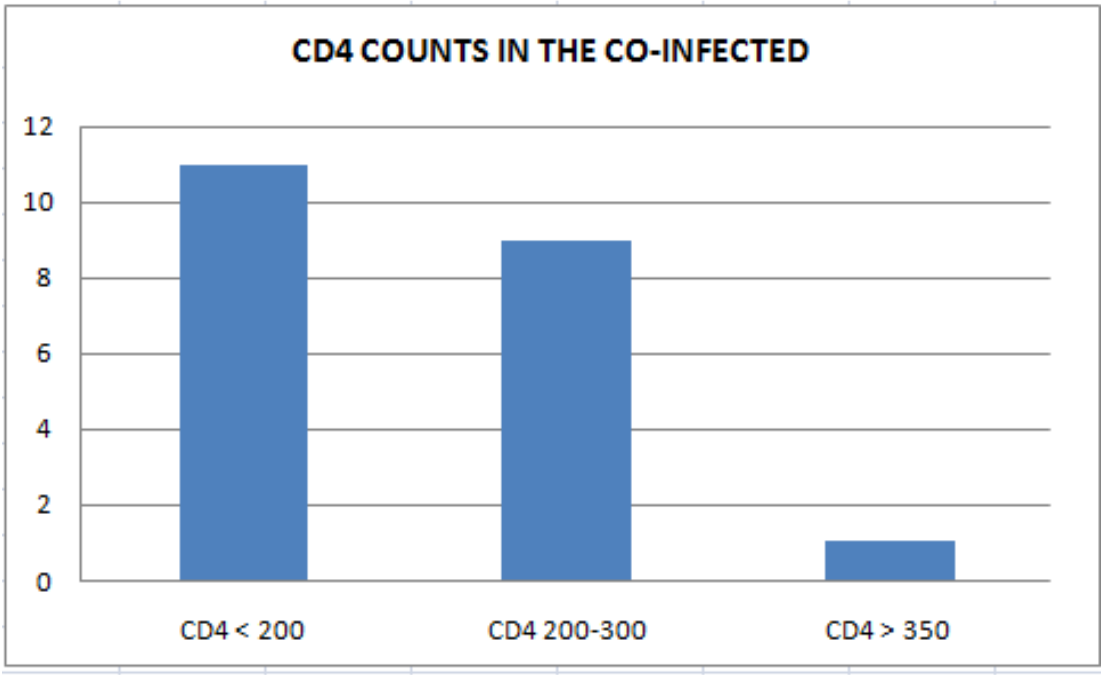

Fig 2: CD4 counts in the *C.neoformans* positive test subjects

Discussion

Cryptococcosis is responsible for an estimated one million cases of meningitis per year, predominantly in HIV infected subjects (Alanio *et al*, 2011). It has been associated with 17% of all deaths among HIV-infected patients in sub-Saharan Africa. With one million cases per year and 700,000 deaths, cryptococcosis is one of the most frequent invasive fungal infection worldwide (Park, 2009).

It has been reported that Infection with *C.neorformans* is thought to be common, however most immunocompetent individuals easily contain the infection (Saha *et al,* 2009). In the absence of effective CD4+ T cell responses, the organism can disseminate and eventually cross the blood –brain barrier to produce cryptococcal meningitis. Positive serum CRAG is relatively high in the study population. This may point in the direction of not just fresh infection with the fungi but also a reactivation of sub clinical pulmonary cyptococcal infection among these individuals. Saha *et al*, 2007 hypothesized that quiescent latent cryptococcal infection may be reactivated in the context of immunosuppression. Studies have reported isolation of the causative agent in pigeons and other healthy birds (Iroakonulo,1999) and in the environment of the present study, bats which are common, are also a source with the causative organisms easily isolated in the rooftops of houses, churches and other public buildings (Mbata,2006). This provides ready sources of infection. In the control group, none of the subjects tested positive suggesting that the immunosuppression in a healthy pregnancy is not sufficient to cause either a reactivation or predisposition to new infection. The combination of the immunosuppression in pregnancy and HIV disease may likely increase the risk of contracting the opportunistic fungal infection. This is suggested by findings in a previous study in this environment which reported 4.2% cryptococcal antigenemia among HIV positive individuals (Chukwuanukwu *et al*, 2013). The result of 13.1% among pregnant women is definitely much higher.

It has been reported by numerous studies that most cryptococcal infections occur in patients with CD4 T cell counts less than 200 cells/ l (Bicanic and Harrison, 2005, Chayakulkeeree and Perfect,2006, Kaur *et al*, 2003, Perfect *et al*,2002, Yoo *et al*,2010). Majority of the infected subjects had CD4 T cell counts lower than 200 cells/ l. However a significant number had CD4 T cell counts between 200 and 300. This may be due to

pregnancy and environmental factors. An individual had CD4 T cell count of 354 cells/ l and had already commenced ART. Literature reveals that while majority of those who present with cryptococcal meningitis are ART naïve, an increasing population are being diagnosed within the first 3 months after ART initiation (Jarvais *et al*,2010, Lawn *et al*,2006).
Cryptococcal antigen can be detected weeks before the onset of symptoms, and those who are asymptomatic but CRAG positive have a high risk of subsequent cryptococcal meningitis and mortality (Rajasingham, 2012). This may be part of the reason why most of the subjects were not down with the disease.

Conclusion
Urgent steps need to be taken to ensure early diagnosis, laboratory strengthening and clinical intervention in this infection, which is most likely under diagnosed, thus contributing to maternal mortality in this vulnerable group.
It is recommended that targeted cryptococcal screening be made a part of baseline tests in HIV positive individuals whose CD4 + T cell counts fall below 200 cells / l, more so in this group who are made more vulnerable due their pregnant state. This is recommended especially since there may be some confusion in symptoms due to already established HIV disease and other opportunistic infections/confounding factors.

Acknowledgement
The authors would like to thank the staff of the Prevention of mother to child transmission (PMTCT) treatment centre and the antenatal clinic staff at Regina-Caeli Hospital Awka, Anambra State.

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