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Detection of *Cryptococcus neoformans* in Cerebrospinal Fluid from Meningitis Patients Associated with HIV/AIDS in Uganda

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Abstract: The significance of *Cryptococcus var. neoformans* meningitis fatality in human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS) patients was confirmed in ten cerebrospinal fluids (CSFs) from Entebbe hospitals in Uganda. All ten CSF specimens were turbid, but on centrifugation, their supernatants were clear and colourless. The protein content of the specimens ranged from 20 to 50mg per 100 ml of CSF (normal range is 10 to 40 mg/100ml). Sugar content ranged between 20 to 60mg per 100ml CFS (normal range 45 to 72 mg/100ml). The white blood cell (WBC) counts in the ten specimens ranged from 15 to 40 WBC/mm³ of CSF (normal range up to 5 WBC/mm³). Gram stain on the deposits, showed capsulated *Cryptococcus (Cr.) neoformans var. neoformans* which were further demonstrated in Odongo-Aginya stain using 5% eosin in 10% formalin mixed in equal volume with 7.5% nigrosin in 10% formalin (Odongo-Aginya *et al.*, 1995). Ziehl Neelsen stain on the deposits, for acid alcohol fast bacilli was negative. *Cr. neoformans var. neoformans* were isolated from all CSF specimens and all patients investigated, died within one week from the time of admission to the hospitals.

Key words: Cryptococcal meningitis, HIV/AIDS patients, hospitals.

The most fatal opportunistic infections in patients with HIV/AIDS in Uganda are those of viruses and fungi (Pedersen *et al.*, 1990). Herpes zoster and Kaposi's sarcoma are viral infections indicating HIV/AIDS and cause severe skin infections and chronic swelling of legs and arms, respectively (Gompel *et al.*, 1992).

Cryptococcus (Cr.) neoformans var. neoformans has been causing death due to cryptococcal meningitis (CM) in Uganda and elsewhere (Mayanja-Kizza *et al.*, 1998; Daniell *et al.*, 1989). *Cr. neoformans var. neoformans* is true pathogenic yeast, unlike *Candida (C.) albicans* (yeast like fungi) (Bruinsma-Adam, 1991). *C. albicans* was not found in the CSFs examined in

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this study but we have been able to isolate *C. albicans* from HIV/AIDS patients with severe stomatitis, throat infections and with sputa (Taylor *et al.*, 1996).

Cryptococcus spp. has been isolated in domestic environment particularly in droppings from chicken and pigeons causing airborne infections to human (Danielle *et al.*, 1989). Rearing pigeons and chickens for domestic consumption is common among Ugandans especially those living in urban areas where high rates of HIV/AIDS also occurs (Joseph *et al.*, 1989). In this study, *Cr. neoformans var. neoformans* has been confirmed as the main causative agent of lethal CM in some inhabitants living with HIV/AIDS in Entebbe town. Although *Cr. neoformans var. gattii*, also cause serious CM in man (Kapend'a *et al.*, 1989; St. Germain *et al.*, 1988), it was not isolated in this study.

Ten CSF specimens, about two to three milliliters, were collected by lumbar puncture in sterile disposable syringe from HIV/AIDS patients admitted in Entebbe hospitals. Four of the patients were females and remaining six were males.

The clinical symptoms recorded on laboratory request forms received together with individual specimens in our laboratory were severe headache with stiff necks. In the laboratory, each specimen was transferred into sterile bijoux bottles and immediately analysed following standard procedures for CSF. Gram stain was used to distinguish different microorganisms in the deposits. Ziehl Neelsen stain was used to study *Mycobacterium (M.) tuberculosis*. Odongo-Aginya stain was used to demonstrate capsule around *Cr. neoformans*.

Sterile deposits from ten CSFs were cultured on blood agar, chocolate agar, and MacConkey agar to isolate other bacteria. Guizotia abyssinica and D-proline, selective and differentiating media for *Cryptococcus spp.*, were used for isolation and identification, respectively (Danielle *et al.*, 1989). Drug sensitivity testing was not carried out due to lack of antifungal discs.

Results on the ten CSF specimens as analysed by standard CSF procedures are summarized in Table 1. All isolates on modified Guizotia abyssinica medium turned its colour into brown at around 27°C room temperature after 7-8 days but did not utilise D-proline under the same condition. This showed that the isolates were all *Cr. neoformans var. neoformans* (Danielle *et al.*, 1989). In Odongo-Aginya stain the capsule surrounding dark *Cr. neoformans var. neoformans* remains as a bright shining unstained structure in pink coloured background produced by mixture of eosin and nigrosin.

All ten cases of *Cryptococcal neoformans* meningitis patients reported in this study, died within a week in Entebbe hospitals. A similar observation was made in Uganda in 1998 (Mayanja-Kizza *et al.*, 1998). The reason was failure to diagnose the disease and administer antifungal chemotherapy earlier. In all cases the patients were treated with antimalaria therapy outside the hospitals. Their CSFs were submitted to our laboratory during the acute

Table 1: Summary of the results of the ten CSF specimens

Specimens N=10	Appearance	WBC/ mm ³	Supernatant	Pro- tein/ mg/%	Sugar/ mg/%	Gram Stains	Ziehl Neelsen stain	Odongo Aginya Stain	Guizotia abyssinica	D-Proline Utilization
1	Turbid	25	Clear	45	50	+	Nil	+	Brown	Nil
2	Turbid	10	Clear	40	60	+	Nil	+	Brown	Nil
3	Turbid	35	Clear	50	55	+	Nil	+	Brown	Nil
4	Turbid	15	Clear	20	45	+	Nil	+	Brown	Nil
5	Turbid	45	Clear	25	35	+	Nil	+	Brown	Nil
6	Turbid	55	Clear	20	50	+	Nil	+	Brown	Nil
7	Turbid	40	Clear	30	58	+	Nil	+	Brown	Nil
8	Turbid	35	Clear	20	37	+	Nil	+	Brown	Nil
9	Turbid	23	Clear	35	30	+	Nil	+	Brown	Nil
10	Turbid	35	Clear	35	20	+	Nil	+	Brown	Nil

Key + = *Cr. neoformans* demonstrated.

stage of the infections. The CSFs were all turbid in appearance but their supernatants were clear and colourless. Except increase WBC from 10 to 55/mm³ and protein 20 to 50 mg/100ml CSF, all other parameters tested were normal. Gram stain and Odongo-Aginya stain were used to demonstrate *Cr. neoformans var. neoformans*. Odongo-Aginya stain was used in this study because it contains nigrosin, which is cheaper and more easily found than India Ink and cryptococcal antigen. Mixture of 7.5% nigrosin and 5% eosin in Odongo-Aginya stain works as well as India Ink for capsul demonstration (Baker and Silverstone, 1978). The 10% formalin in Odongo-Aginya stain has an additional advantage of fixing and killing all microbes including HIV/AIDS virus in the specimens thus it reduces accidental risk of infections when working with such specimens. In Uganda, scarcity of cryptococcal chemotherapy coupled with the cost of the treatment whenever available have resulted in some deaths of HIV/AIDS patients infected with *Cr. neoformans var. neoformans*. From these recorded and unrecorded cases of CM in HIV/AIDS in Uganda, it appears that this opportunistic infection is one of the most fatal ones without cure. Early diagnosis of *Cr. neoformans var. neoformans* and treatment in HIV/AIDS patients in Uganda and elsewhere would enable some of them to survive longer (Mayanja-Kizza *et al.*, 1998). *Cr. neoformans var. neoformans* infections are treatable and can be cured. The social and environmental risk of exposure of each individual determines the acquisition of the *Cr. neoformans var. neoformans*.

These observations, which is the first of this kind in Entebbe, suggest that, in Uganda and other developing countries, HIV/AIDS patients with symptoms described above should

have their CSFs taken for CM study. This will enable the commencement of early treatment on CM positive patients (Mayanja-Kizza *et al.*, 1998). Therefore Ministries of Health should provide appropriate medicaments for cryptococcosis in all hospitals at subsidised cost.

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