

PREVALENCE OF CRYPTOSPORIDIOSIS IN HIV-INFECTED PATIENTS IN KAJANG HOSPITAL, SELANGOR

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Abstract. A total of 66 fecal specimens obtained from patients infected with human immunodeficiency virus (HIV) from Kajang Hospital were screened for *Cryptosporidium* oocysts. The fecal specimens were concentrated using the formalin ethyl acetate concentration technique, stained with modified Ziehl-Neelsen and confirmed with immunofluorescence stain. It was established that 2 (3.0%) were positive for *Cryptosporidium*. The two cases involved a Chinese local man (with diarrhea) and an Indonesian foreigner (without diarrhea). A higher index of suspicion for clinical cryptosporidiosis in HIV patients, including those with chronic weight loss with or without diarrhea, is recommended. In addition, laboratory testing for *Cryptosporidium* in HIV-infected patients is highly recommended in order to have a better understanding of the epidemiology and management of the disease in Malaysia.

INTRODUCTION

Over the past decade, the role of *Cryptosporidium* as an agent of human diarrhea has been redefined from that of a rare opportunist to a pathogen with worldwide distribution and the potential for significant morbidity and mortality. Lately, the strong association between cases of cryptosporidiosis and immunodeficient individuals (such as those with AIDS) brought *Cryptosporidium* to the forefront as an ubiquitous human pathogen (Smith *et al*, 1998).

Presently, the increasing population of immunocompromized persons and various outbreaks through infection by waterborne *Cryptosporidium* oocysts (often in drinking water) have placed an even greater emphasis on this pathogen. To date, there is no proven effective drug therapy for this parasite, and, in part due to its inability to grow well *in vitro*, the human immune response to cryptosporidial infection remains poorly understood.

The spread of human immunodeficiency virus (HIV) infection in Malaysia has risen significantly since the early 1980s. As of 31st December 2001, the Ministry of Health Malaysia reported that there were 44,272 cases of HIV infections in Malaysia with an average of 5,000 new infections detected per year (Ministry of Health, Malaysia, 2002). Opportunistic infections, to which HIV infected patients are

susceptible, comprise only a minority of the large number of parasites capable of causing human disease. Intestinal parasites that have exploited the immunological defect in HIV infected patients include *Cryptosporidium* species (Zumla and Crofts, 1992).

Cryptosporidium, a small, obligate intracellular parasite, has emerged as an important cause of chronic life-threatening diarrhea (Das *et al*, 1993), causing prolonged and cholera-like diarrhea in HIV infected patients (Petersen, 1992). However, in immunocompetent persons, it may cause a short-term diarrheal illness that resolves spontaneously. The route of transmission has been associated with fecal-oral, as well as through drinking contaminated water, person-to-person spread and contact with infected animals (Ng and Shekhar, 1993; Morgan and Thompson, 1998).

A prospective long-term study from Europe suggested that 3-4% of those with HIV will have cryptosporidiosis and that an equal number will develop it later in the course of their disease (Pedersen *et al*, 1996). However, this parasite has been identified in up to 46% of HIV patients world-wide in other studies (Bonilla *et al*, 1992).

In Malaysia, the problem of cryptosporidiosis among HIV patients has not received much attention, as evidenced by the lack of reports. This could possibly be due to underdiagnosis of the disease, or patients being screened only when they reach the AIDS stage with chronic, persistent diarrhea or when symptomatic treatment fails. Another contributing factor could be a lack of emphasis on this diagnosis, as there remains no specific cure for the disease apart from antiretroviral therapy. The first reported study among HIV positive intravenous drug users (IVDU) in Malaysia was

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conducted in 1994 indicating high prevalence (23%) of cryptosporidiosis (Kamel *et al*, 1994). Since then there has not been any other report.

The aim of this present study is to determine the prevalence of cryptosporidiosis among HIV infected patients in a hospital setting. The findings will hopefully lead to better understanding and management of cryptosporidiosis in HIV infected patients in Malaysia.

MATERIALS AND METHODS

A total of 66 HIV infected patients from Kajang Hospital, Selangor were included in this study. A single stool specimen was collected from each patient between April and July 2004. The consistency of the stool specimens was graded by categories (soft, watery, etc) while waiting to be processed.

Stool specimens were concentrated using the formalin-ethyl acetate concentration technique and stained with modified Ziehl-Neelsen (acid-fast stain). Slides were examined under 400x. *Cryptosporidium* oocysts appeared as bright rose-pink spheres ($5 \pm 1 \mu\text{m}$) on a pale green background. All slides positive for *Cryptosporidium* using the Ziehl-Neelsen staining method were recorded.

The presence of *Cryptosporidium* oocysts in the positive specimens were then confirmed using the immunofluorescence technique, which is more specific and sensitive. In the immunofluorescence technique, specimens were stained with the commercially available fluorescein isothiocyanate (FITC)-labeled anti-*Cryptosporidium* oocysts mAb (Crypto-a-Glo kit, Waterborne, Inc, New Orleans, USA), which stains the oocyst wall bright apple green and 4', 6-diamidino-2-phenylindole (DAPI) stain (Sigma Chemical, Missouri, USA), which stains the four nuclei sky-blue. Slides were viewed under 400x epifluorescence microscope (Olympus BX52, Japan) with wide band excitation color separation filter (exciter 460-490 nm, barrier 550 nm) to detect FITC stain, a wide band UV filter (exciter 330-335 nm, barrier 420 nm) for DAPI stain and Nomarski-DIC optics to observe the internal structures of *Cryptosporidium* oocyst (about 4 to 6 μm in diameter). Positive control slides containing oocysts and negative control slides were included in the analysis.

RESULTS

A total of 66 stool specimens were obtained from HIV infected patients admitted to Kajang Hospital. The range of patients' ages was 29-56 years. They consisted

of 59 (89.4%) males and 7 (10.6%) females. Their various ethnicities were: Malay (47; 71.2%), Chinese (9; 13.6%), Indian (8; 12.1%), and others (2; 3%). Only 6 (9.1%) of the stool specimens were watery, while soft and hard stool forms were 31 (47%) and 29 (43.9%), respectively.

Out of 66 stool specimens screened for *Cryptosporidium* oocysts, only 2 (3.03%) were positive for cryptosporidiosis. The profiles of the two positive patients with cryptosporidiosis were as follows:

Case 1

LKH was a 37-year old Chinese man who was admitted into Kajang Hospital in a very ill state. He was known to have HIV but was not on any antiretroviral therapy. His baseline CD4 counts were unknown. He was also known to have pulmonary tuberculosis (TB) and was on anti-TB medications. Examination revealed a weak, cachectic, dehydrated and hypotensive patient. While in the ward, he was noted to have diarrhea. Laboratory diagnosis later detected *Cryptosporidium* oocysts in his stool sample with a concentration of 1.1×10^6 oocysts per gram. Unfortunately the patient succumbed to his illness 36 hours after admission.

Case 2

SS is a 29-year old Indonesian man. He was known to have HIV infection but was not on antiretroviral therapy. His CD4 count was 37 cells/mm³. He also had pulmonary TB and was on anti-TB medications. He presented with a one-month history of reduced oral intake, reduced appetite and vomiting. He did not have diarrhea. He was cachectic, weak, dehydrated and had a low grade fever. He also had oral cavity ulcers which healed with treatment. Laboratory diagnosis later detected *Cryptosporidium* oocysts in his stool with a concentration of 3.6×10^6 oocysts per gram. He has since been deported back to Indonesia.

DISCUSSION

The prevalence of cryptosporidiosis in Malaysian HIV-infected hospital patients as revealed in this study was 3.03%. This finding is similar with the results obtained by Sorvillo *et al* (1994) and Pedersen *et al* (1996), who reported 3-4% of those with HIV have cryptosporidiosis. Epidemiological surveys carried out in various parts of the world revealed that the prevalence rate of cryptosporidiosis was anywhere from 3-50% (Bonilla *et al*, 1992; Pedersen *et al*, 1996). A number of studies indicated that the prevalence of cryptosporidiosis in HIV-infected patients was in the range of 15% or below (Florez *et al*, 2003).

In Brazil, the occurrence of *Cryptosporidium* infection in 52 HIV/AIDS patients was found to be 5.8% using safranin/methylene blue, modified trichrome staining techniques and enzyme immunoassay (EIA) (Silva *et al*, 2003). In another study, also in Brazil, 9.33% of samples were positive for *Cryptosporidium*, with higher frequency of cases in male patients from 20 to 50 years old (Ribeiro *et al*, 2004).

A study among 156 HIV-infected Thai patients who had acute diarrheal illness showed that 12.8% had cryptosporidiosis (10.0% in males and 19.1% in females) (Saksirisampant *et al*, 2002). However, in Nigeria, a study carried out on 161 HIV-infected patients with chronic diarrhea revealed no cryptosporidium oocysts or any other acid-fast organism in stool. It was concluded that intestinal cryptosporidiosis is not common in HIV-infected patients with chronic diarrhea in Enugu. Nonetheless, more studies are needed to further confirm this trend (Nwokediuko *et al*, 2002).

The two patients with cryptosporidiosis in this study had differing presentations. The first had classical symptoms of severe diarrhea to a point where he was severely dehydrated and cachectic by the time he was admitted. In fact, cryptosporidiosis was likely to be the cause of his demise.

The importance of *Cryptosporidium* as a widespread cause of diarrhea is well recognized. Reported prevalence of cryptosporidiosis associated with diarrhea in Asia ranged from 3.2% in Thailand, 3.6% in China, 10.0% in Indonesia and 13.3% in India (Katsumata *et al*, 1998). A study in patients with HIV infection in South Italy showed that 21.5% of patients with diarrhea and 3.4% of patients without diarrhea had cryptosporidiosis. A significant ($p < 0.001$) correlation was observed between *Cryptosporidium* infection and the presence of diarrhea (Brandonisio *et al*, 1999).

The second patient in this study had a subclinical presentation. The symptoms of loss of appetite, reduced oral intake, weight loss, vomiting, and low grade temperature may well have been due to cryptosporidiosis, but there was no history of chronic diarrhea. His CD4 count was also very low (37 cells/mm³). Flanigan *et al* (1992) reported that 87% of patients with CD4 counts of less than 140 cells/mm³ who have cryptosporidiosis will develop persistent disease. Unfortunately, the progress of this patient could not be tracked since he was deported back to his homeland, Indonesia.

Occurrence of asymptomatic (no signs of diarrhea)

carriers of *Cryptosporidium* in HIV patients was not surprising. High occurrences (23%) of *Cryptosporidium* in asymptomatic HIV positive IVDU drug users have been reported by Kamel *et al* (1994). Ravn *et al* (1991) also found the occurrence of asymptomatic carriers of *Cryptosporidium* to be common among AIDS patients. The presence of *Cryptosporidium* oocysts in asymptomatic patients noteworthy because they can act as important reservoirs for the organism and might be a potential source of infection.

In Malaysian hospitals, HIV infected patients are normally worked up for cryptosporidiosis when the CD4 levels are low and the patients present with chronic, persistent diarrhea. It would be advisable to recognize that cryptosporidiosis can present with just chronic weight loss and other nonspecific symptoms devoid of diarrhea. Hence, a higher index of suspicion for clinical cryptosporidiosis in HIV patients, including those with chronic weight loss with or without diarrhea, is recommended. Additionally, laboratory testing for *Cryptosporidium* in HIV infected patients is highly recommended in order to have a better understanding of the epidemiology and management of the disease in Malaysia.

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