CASE REPORT

CYCLOSPORA INFECTION IN AN HIV INFECTED PATIENT WITH ULTRASTRUCTURAL STUDY

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Cyclospora cayetanensis, a coccidian protozoa of the phylum Apicomplexa, was first described in 1979 by Ashford and named by Ortega *et al* in 1992. The organism in fresh stool appears as a spherical cyst containing refractile globules and measuring 8-10 μm in diameter. The mature oocyst is composed of two sporocysts each containing two sporozoites (Ortega *et al*, 1993). Under ultraviolet light the oocyst wall shows bright blue autofluorescence (Long *et al*, 1991). The protozoan parasite had been reported as an enteric pathogen causing chronic watery diarrhea in both healthy and HIV infected persons (Soave *et al*, 1986; Hart *et al*, 1990; Long *et al*, 1990; Long *et al*, 1991; Shlim *et al*, 1991). However, asymptomatic excreters of the oocysts can be found (Ashford 1979; Pollok *et al*, 1992). The present case is the first reported case of *Cyclospora* infection found in Thailand in an HIV infected patient. Ultrastructural features of the organism are also described.

A 28-year-old Thai male who was diagnosed as having AIDS at Bamrasnaradura Infectious Diseases Hospital since late 1993, was admitted for investigations of chronic watery diarrhea in June 1994. He presented with a one-month history of episodic watery diarrhea and had been passing stools up to 5 times a day. He had lost 8 kg in weight. His illness was associated with nausea and vomiting. He had no history of fever, chills, abdominal pain, or any noticable blood or mucus in his stool. Eight days prior to admission he received norfloxacin 800 mg per day orally for 5 days, but there was no clinical improvement. He was found to have positive serology for HIV antibodies since 1993. Although he admitted to having some extramarital sexual contacts in the past, he denied any history of intravenous drugs use or homosexual behavior.

Physical examination revealed temperature 36.5°C, pulse rate 86/minute, respiration rate 20/minute, blood pressure 100/60 mmHg and body weight 38 kg. He was cachectic but alert. Oral thrush was noted with multiple lymphadenopathy found in both cervical regions. There were neither hepatosplenomegaly nor abdominal masses. Other findings were unremarkable.

Laboratory data showed a white cell count of 6,500/mm^3, with 82% neutrophils, 2% band forms, 15% lymphocytes, and 1% eosinophil. The hematocrit was 38% and the platelet count was 236,000/mm^3. The CD8 : CD4 ratio was 1.50 and his absolute CD4 count was 145/mm^3. The chest roentgenogram was unremarkable. A fecal specimen was negative for occult blood and leukocytes. The stool culture yielded no pathogenic bacteria.

Gastroscopy and gastric biopsy were performed and revealed no abnormality. Duodenoscopy showed edematous mucosa and histologic sections contained increased numbers of plasma cells and lymphocytes infiltration in the lamina propria as well as villus blunting. Colonoscopy revealed edematous mucosa of the ascending colon. The histologic sections contained only non-specific chronic colitis. *Cyclospora* was not seen by light microscopy.

Electron microscopic study of duodenal biopsied specimen revealed numerous parasites of various shapes and sizes, seen in the cytoplasm of the mucosal cells. They were confined within the vacuoles and often seen in groups of three to eight parasites (Figs 1 and 2). Electron microscopic study of stomach and colon revealed no evidence of *Cyclospora*.

One of the cervical lymph nodes was removed for pathological examination. The sections demonstrated foci of necrotizing inflammation with multiple encapsulated budding yeasts. The histopathological diagnosis was cryptococcal lymphadenitis.

Fig 1–A group of parasites are demonstrated within parasitophorous vacuoles.

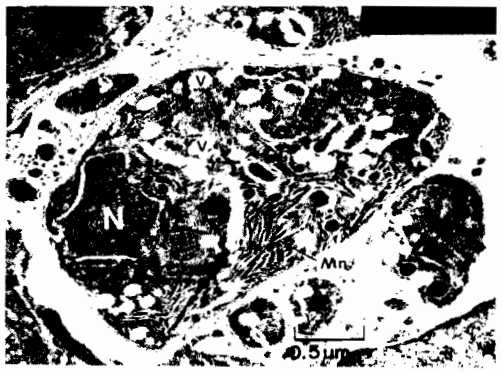

Fig 2–At higher magnification, the fine structures of sporozoite are clearly seen; nucleus (N), vacuole (V), and micronemes (Mn).

On the first day of admission, the patient still passed greenish watery stools 3 times a day and oral rehydration therapy was administered. Fecal specimen preserved in 10% formaldehyde was sent to the Department of Parasitology, Faculty of Medicine Siriraj Hospital for examination. Stool microscopy revealed numerous spherical organisms containing refractile greenish tinge granules. The size of the oocysts varied from 8 to 10 μm in diameter. After formalin-ether concentration, the striking lower number of organisms was noted. With the modified acid-fast stain, these round bodies stained deep red but some remained unstained glassy, wrinkle spheres. The organisms were refractory to Giemsa and Gomori methenamine-silver stains. Under UV light, the walls of the organisms showed bright blue autofluorescence. They were identified as *Cyclospora*.

Cotrimoxazole one tablet daily and cotrimazole troches were prescribed for *Pneumocystis carinii* pneumonia prophylaxis and oral candidiasis respectively. Cryptococcal lymphadenitis was treated with fluconazole 400 mg orally per day. Lumbar puncture was suggested by the attending physician but the patient refused the investigation. His diarrhea disappeared on the third day after admission, he then was discharged. Two weeks later, he was seen again at the outpatient department and he had no complaint of diarrhea. He also had gained 3.2 kg in weight. For the next 3 months of follow-up examination, he remained asymptomatic and continued to gain weight. Subsequent stool examinations showed no parasites.

During the past few years, there have been several reported cases of chronic diarrhea associated with a granule-containing spherical body measuring 8-10 μm in diameter. It was initially reported in 2 patients with diarrhea and one carrier in Papua New Guinea (Ashford, 1979). In 1986, the organism was identified in diarrheic stools from 4 immunologically competent hosts who visited Haiti and Mexico and was described as resembling a coccidian body or a fungal spore (Soave *et al*, 1986). The organism had previously been referred to as a large *Cryptosporidium*, an alga-like organism, a cyanobacterium-like body or a coccidian-like body (Long *et al*, 1990; Long *et al*, 1991; Shlim *et al*, 1991). The name *Cyclospora cayetanensis* was proposed on the basis of its growth feature *in vitro*, staining characteristics, its autofluorescence feature under ultraviolet illumination, and its distinct features on electron microscopic study (Ortega *et al*, 1992; 1993). It was classified as a coccidian protozoa in the phylum Apicomplexa.

The oocysts from freshly passed stool are unsporulated. It takes approximately 7 days in water or 5-13 days in potassium dichromate solution to produce two sporocysts, each containing two sporozoites (Long *et al*, 1991; Ortega *et al*, 1993). The mature oocysts of *Cyclospora* are different from those of *Cryptosporidium*, the latter are smaller in size measuring 4-6 μm in diameter and have four naked sporozoites within each oocyst. With the modified acid-fast staining property, *Cyclospora* could be easily confused with *Cryptosporidium* unless organism sizes are measured. The autofluorescence of *Cyclospora* oocysts appears as bright blue circles in stool smear under ultraviolet light. This technique is a sensitive and reliable method for detection of the organisms but not for *Cryptosporidium* oocysts (Long *et al*, 1991).

Cyclospora infection has been reported worldwide in both immunocompetent and immunocompromised patients. Almost all infected persons suffer from watery diarrhea. The diarrheal illness is characteristically prolonged and intermittent. The associated symptoms include abdominal cramps, weight loss, nausea, vomiting, flatus, fatigue, anorexia, fever and myalgia (Long *et al*, 1991; Shlim *et al*, 1991). The parasites can be found from stool samples, jejunal juice or duodenal aspirates (Bendall *et al*, 1993; Connor *et al*, 1993). The intracytoplasmic cresent-shaped organisms were found by electron microscopic examination from jejunal biopsy specimens (Bendall *et al*, 1993), but not from distal duodenal biopsies (Connor *et al*, 1993).

In this case, the patient with AIDS suffered from chronic watery diarrhea. *Cyclospora* was diagnosed as the causative agent from stool microscopy and electron microscopy of duodenum. He was previously treated with norfloxacin for 5 days and became asymptomatic on day 10 after treatment. The follow-up stool examination was negative for parasites. The efficacy of the antibiotic treatment was difficult to assess because of the self-limiting nature of the diseases, although in immunocompromised patients its course tended to be prolonged. Shlim *et al* (1991) suggested that no apparent benefit resulted after treatment with norfloxacin, tinidazole, quinacrine, nalidixic acid and diloxanide furoate. Gascon *et al* (1993) reported that one patient recovered after ciprofloxacin administration and others recovered without treatment. Cotrimoxazole therapy was noted to be effective in 5 cases reported by Madica *et al* (1993). In this case, norfloxacin and oral rehydration therapy were thought to be the reason for his clinical improvement, and the low dose cotrimoxazole used for prophylaxis of *Pneumocystis carinii* pneumonia might not effect the course of illness. The controlled clinical trial of antiprotozoal drugs against *Cyclospora* should be further investigated.

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