Isospora belli infection associated with Chronic Cholecystitis and Sclerosing Cholangitis in Immunocompetent Host : A Case Report

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Abstract : ***Isospora belli (I. belli)*** **is a spore-forming protozoa that primarily infects enterocyte. The clinical manifestation may vary from asymptomatic to self-limited diarrhea in healthy persons. Rarely, in the normal hosts,[5] chronic persistent or intermittent symptom may continue for many years.[1,2] Treatment with cotrimoxazole is usually effective in both normal patients[19] and patients with AIDS[20,21] Nevertheless, relapse is common and is believed to be associated with the presence of extraintestinal infection.[3,4,17] Few cases of extraintestinal *I. belli* infection have been documented in patients with AIDS,[3-5] and one case in an immunocompetent patient.[24]**

This report presents a unique case of isosporiasis in a 58-year-old anti-HIV negative Thai female suffering from chronic recurrent diarrhea due to *I. belli*, despite two episodes of complete 4-week cotrimoxazole and another course of nitazoxanide. Abdominal computer tomographic examination and ultrasonography identified sclerosing cholangitis and chronic cholecystitis. Histologic examination of the cholecystectomy specimen recovered schizonts and merozoites in the parasitophorous vacuoles located in gallbladder epithelium.

เรื่องย่อ : การติดเชื้อ *Isospora belli* ในโรคถุงน้ำดีอักเสบและท่อทางเดินน้ำดีอักเสบ : รายงานผู้ป่วยหนึ่งรายที่มีภูมิคุ้มกันปกติ

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รายงานผู้ป่วยหญิงไทย อายุ 58 ปีที่มีภูมิคุ้มกันปกติหนึ่งราย มาโรงพยาบาลด้วยเรื่องถ่ายอุจจาระร่วงเรื้อรังมานาน หลังจากการตรวจอุจจาระ พบเชื้อ *Isospora belli* ผู้ป่วยได้รับการรักษาด้วยยาและอาการดีขึ้น สี่เดือนต่อมาอาการถ่ายอุจจาระร่วงกำเริบ ร่วมกับการอักเสบของท่อทางเดินน้ำดีอักเสบ และถุงน้ำดี การตรวจท่อทางเดินน้ำดีและถุงน้ำดีพบว่ามีการติดเชื้อ *I belli* รายงานนี้แสดงลักษณะทางจุลพยาธิวิทยาและการศึกษาด้วยกล้องจุลทรรศน์อิเล็กตรอนของเชื้อ *I belli* ในท่อทางเดินน้ำดี และถุงน้ำดี

CASE REPORT

The patient was a 58-year-old Thai female presented with carpopedal spasm and chronic intermittent watery diarrhea. Serum electrolytes found hypocalcemia (serum total calcium level 5 mg%) and stool examination recovered oocysts of *I belli*. Clinical improvement was noted after calcium supplement and four weeks of cotrimoxazole. Four months after the complete course of medication, the diarrhea with *I belli* relapsed. Anti-HIV status was negative with CD4 count of 640 cells/ μL. Repeated dose of cotrimoxazole failed to cure. The medication was shifted to nitazoxanide. However, sporocysts still persisted in the stool after therapy ended. Endoscopic Retrograde Cholangio Pancreatography (ERCP) showed dilated biliary tree from the papilla to the intrahepatic duct. Papillar biopsy reported as inflamed chronic cholangitis with isosporiasis. Liver function tests were total bilirubin 0.2 mg%, direct bilirubin 0.1 mg%, SGOT 58 U/L, SGPT 48 U/L, alkaline phosphatase 175 U/L, Albumin 3.2 mg% and Globulin 3.6 mg%. Repeated ERCP showed dilated biliary tree from the papilla to the intrahepatic bile duct without filling defect. No common bile duct stone was seen. Cholecystectomy was performed. The gallbladder and attached cystic duct measured 8.3x2.5x1.8 cm. The serosal surface appeared unremarkable. The wall measured 0.4 cm in maximal thickness. The mucosa displayed diffuse papillary surface with prominent finger-like projection at fundus and neck. (Figure 1A,B) The cystic duct measured 1.6 cm in length and 1.3 cm in circumference. The lumen contained greenish brown mucoid sticky bile without stone. Microscopic examination disclosed tall mucosal folds with crypt hyperplasia, elongated fibrovascular cores and occasional branching. They were lined by tall, columnar epithelial cells with regular basal nucleus admixed with a few mucus-secreting cells. Intense infiltration by eosinophils and plasma cells was noted in the lamina propria and epithelial layer of these prominent papillary folds. (Figure 2) Various intracellular stages of the schizogony and gametogony were observed. The infected cells contained parasites in membrane-bound, parasitophorous vacuoles, isolating them from host cells hence enhancing visualization in tissue section. (Figure 3A) The intracellular presentation of single organism was typically schizont, while the multiple organisms were merozoites. Microgametocyte with many peripheral microgamates was focally seen. The macrogametocyte had basal position of the organism, prominent nucleus and nucleolus. (Figure 3B) This feature was found in the intracytoplasm of gallbladder epithelium and rarely seen in macrophage residing in lamina propria.

Transmission electron microscopic examination was done on fragments of formalin fixed tissue and many elongated intracellular parasites were observed in longitudinal and cross sections. The parasites were surrounded by a parasitophorous vacuole filled with a dense granular material in the outer portion and a loose dense fibrillar material around the parasites inside the cytoplasm of epithelial cells or macrophages. (Figure 4) The parasites showed a distinct spherical nucleus and unbound crystalloid bodies toward the rostral end. (Figure 5,6)

DISCUSSION

I belli is an intestinal spore-forming protozoa, belonging to family Eimeriidea, suborder Eimeriina, subclass Coccidia and phylum Apicomplexa. Isosporiasis is an intestinal infection by one of the three isosporas, *I hominis, I natalenis* and *I belli*. Only *I belli* has been identified in humans.[6] Isospora is found predominantly in the tropical and

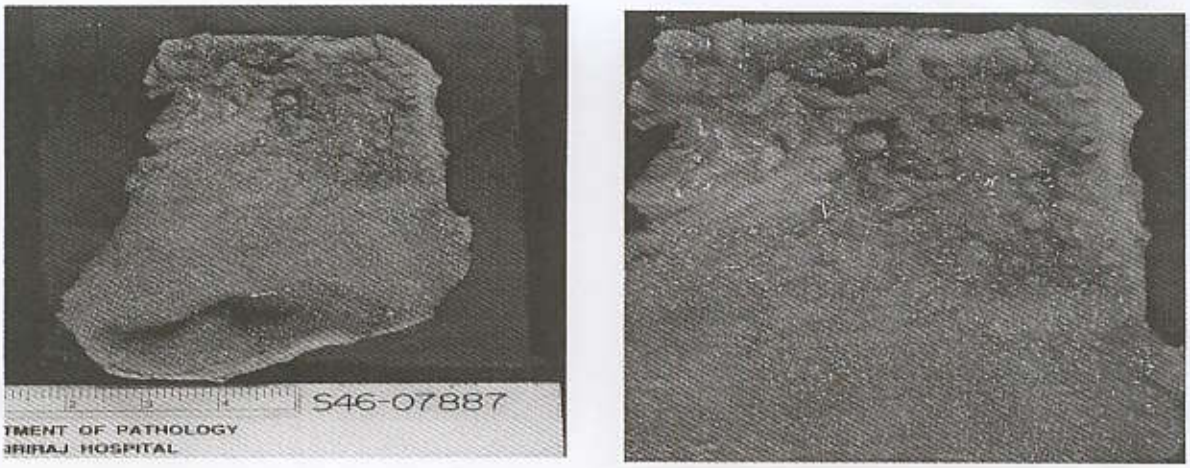

Figure 1. (A) The gallbladder and attached cystic duct measured 8.3x2.5x1.8 cm. The serosal surface appeared unremarkable. The wall measured 0.4 cm in maximal thickness.
(B) The mucosa displayed diffuse papillary surface with prominent finger-like projection at fundus and neck.

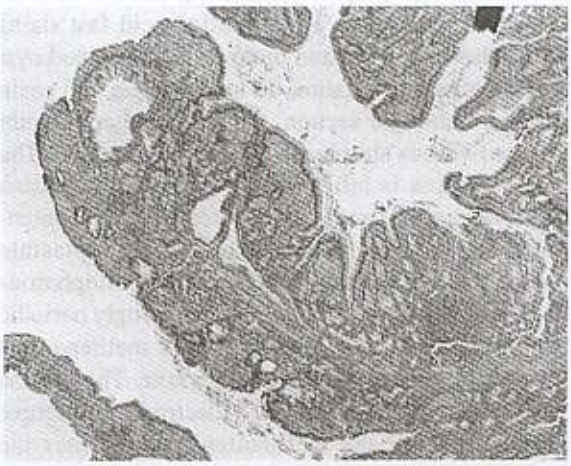

Figure 2. The gallbladder disclosed tall mucosal folds with fibrovascular cores and occasional branching. They were lined by tall columnar cells with regular basal nuclei admixed with a few mucus secreting cells [Hematoxyline & Eosin x10]

subtropical areas, especially in South America, Africa and Southeast Asia.[7,8] The prevalence of human isosporiasis is unknown.[9] In patients with AIDS, prevalence of *I belli* ranges between 5 to 15%[10-12] Transmission occurs via environmental exposure and fecal contamination (food and water); direct person-to-person transmission is yet unproven.[9]

After ingestion of the infective oocyst, through the action of bile and trypsin at distal duodenum and jejunum, the sporozoites emerge and penetrate the cytoplasm of enterocytes where they undergo both sexual and asexual reproduction. After multiple cycles of schizogony, gametocytes form. A zygote is produced through sexual reproduction. A cyst wall develops around the zygote, forming the oocyst.[13] To be infectious, the excreted oocyst must be sporulate, a process that can take up to 48 hours and requires oxygen and temperatures below 37 °C.[9] Because *I. belli* develops only in human, details of the intracellular development within enterocytes are not as completely known.[13]

The clinical illness resulting from isosporiasis depends on the competency of the host's immune system. In immunocompetent hosts, the illness is indistinguishable from other non-inflammatory intestinal infections, such as giardiasis, cryptosporidiasis, cyclosporiasis, and enterotoxigeric *Escherichia coli* infection. After a one-week incubation, a self-limited profuse watery diarrhea without blood could last for three weeks, and may be accompanied by malaise, anorexia, weight loss and abdominal cramps. Oocyst shedding may persist for

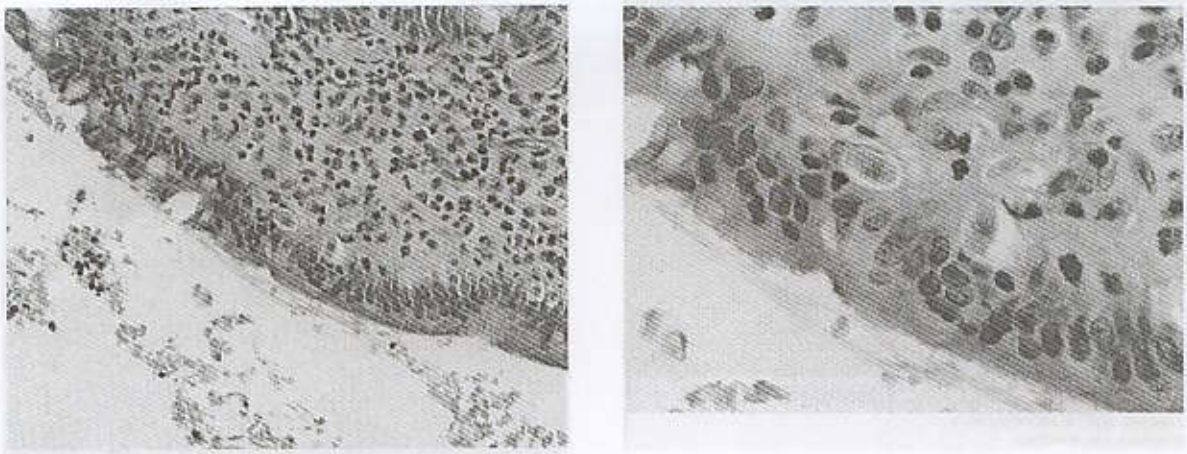

Figure 3. (A) Intense infiltration by eosinophils and plasma cells in lamina propia. Parasites in the parasitophorous vacuoles located in basal gallbladder epithelial cells (H&E) x 400).
(B) Macrogametocytes with prominent nucleus (H&E x 1000).

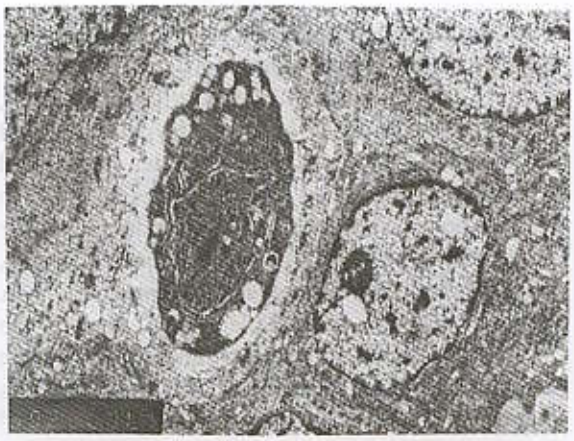

Figure 4. The parasites were surrounded by a parasitophorous vacuole filled with a dense granular material in the outer portion and a loose dense fibrillar material around the parasites inside the cytoplasm of epithelial cells or macrophages. (x 15,400)

between several weeks and four months during convalescence.[14,15]

Diagnosis is based on the identification of the oocysts in fecal specimens.[8] The oocyst contains 2 sporocysts, measures 20-33 mm long by 10 to 19 mm wide. They taper at the ends, have a double-layer refractile wall. The modified acid fast stains sporoblasts deep red and outlines the unstained cyst wall by dye precipitation. In hematoxylin and eosin stained histologic section, the intestinal epithelium showed villous atrophy with crypt hyperplasia. The lamina propia is infiltrated by eosinophils, plasma cells, neutrophils and lymphocytes. All developmental stages are identified in an intracytoplasmic membrane bound vacuole, so-called parasitophorous vacuoles. The typical schizonts are strongly periodic acid-Schiff positive. The Gomori's methenamine silver stain showed all stages of parasite. The Giemsa stain gives the best contrast.[9] Electron microscopic examination of the merozoites demonstrates the apical complexes that include polar rings, spirally coiled filaments (conoid), longitudinally oriented electron-dense organelles in the anterior body (minonemes), electron-dense tubular or saccular organelles (rhoptries), and subpellicular tubules.

The specific experimental evidence explaining the pathogenesis of diarrhea in intestinal spore-forming protozoal infections (cryptosporidia, microsporidia, isospora and cyclospora) is limited and only pathologic studies on cryptosporidiosis were well established.[25] The pathogenesis of isosporiasis has not been determined but may be due to cell damage from direct consequence of parasite

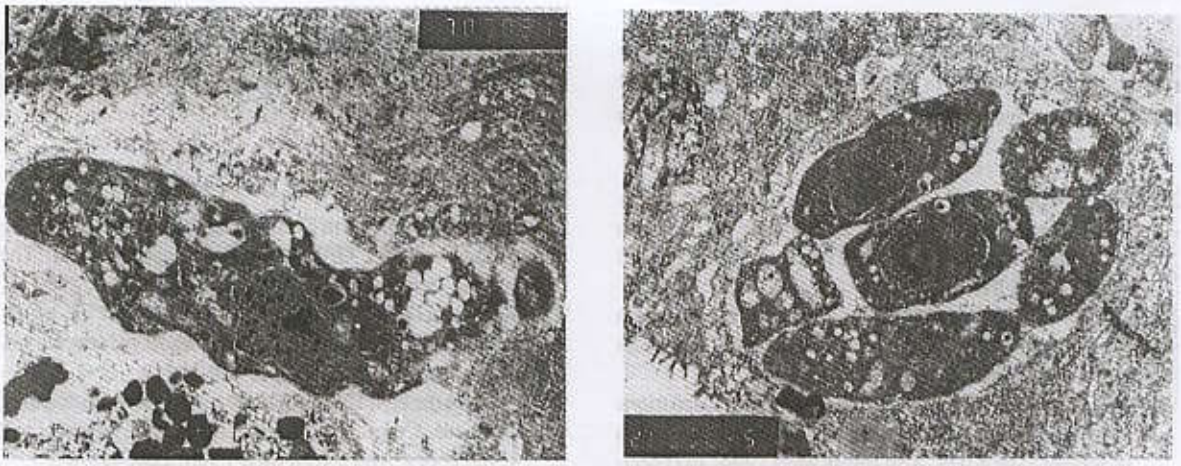

Figure 5A,B. The parasites showed a distinct spherical nucleus and unbound crystalloid bodies toward the rostral end. (x 15,400)

invasion, cell-mediated inflammation, or proteins and oxidants released from mast cells. Morphologic changes include villous shortening or flattening, crypt hyperplasia, and increased numbers of leukocytes (particularly eosinophils and plasma cells) in lamina propria and epithelium. Heavy infection with parasites is associated with the severity of structural alterations of the enterocytes, including changes in the shape of the cell from columnar to cuboidal to pleomorphic, atrophic of microvilli, swelling of mitochondria, Golgi apparatus and endoplasmic reticulum with accumulation of lysosomes and lipid vacuoles.[19] These histopathologic changes are more extensive than just infected cells and suggest that the parasite releases a toxic agent or stimulates a hypersensitivity reaction.[9] Invasion and ulceration do not occur. The chronic isosporiasis with severe functional abnormalities may result in marked steatorrhea and decreased fat absorption.[16]

The recommended treatment of trimethoprim sulfamethoxazole (160/800 mg) four times a day for ten days followed by three times a day for three weeks has proved effective.[22] Response is usually within 48 hours. It is even effective in treating the recurrences, which may occurs up to 50% after one week off therapy.[9] Anecdotal reports suggest that diclazuril, roxithromycin, nitazoxamide and combination of albendazole and ornidazole may be effective.[23]

Extraintestinal *I belli* infection has been documented in AIDS patients at lymph node, liver and spleen histiocytes.[4,5] The profound immunodeficient state possibly in conjunction with coinfection and ulceration of intestinal mucosa, promotes access of *I belli*, into lymphatic and vascular spaces.[4] As with other intestinal spore-forming protozoa, *I belli* is capable of involving contiguous epithelial surfaces in both immunocompetent and immunocompromised host, especially in heavy infection.[3,16] Infection in the biliary tract may result from subsequent spread from periampullary area retrograde into gallbladder, resulting in acalculous cholecystitis and cholangitis.[3,24]

The intractable diarrhea due to *I belli* despite appropriate medical therapy in this patient indicated treatment failure. The cause of relapse of *I belli* infection in immunocompetent and AIDS patients is probably the extraintestinal tissue cysts which are most likely dormant and not susceptible to anticoccidial treatment. Another possibility, documented by Bialek R in 2001 about chronic biliary isosporiasis in 60-year-old immunocompetent patient with failure to respond to oral cotrimoxazole prophylaxis and oral nitazoxanide treatment, was the subtherapeutic level of nitazoxanide in plasma and bile due to severe malabsorption, leading to ineffective treatment. In the very same case the intravenous cotrimoxazole stopped the shedding of *I belli* oocysts in bile within 5 days thus excluding the initially suspected resistance to cotrimoxazole.[24]

In conclusion, the patients diagnosed with intestinal isosporiasis who experience multiple relapses after treatments with standard medication are recommended to further investigations for evidence of extraintestinal infection, especially in the biliary tract and gallbladder.

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