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Case Report

Severe Diarrhea Due To *Cystoisospora belli* Infection in an HTLV-1 Woman

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Abstract

Cystoisospora belli, formerly *Isospora belli*, as an opportunistic infection agent, is seen in immunocompromised patients like HTLV-1. We describe here cystoisosporiasis in an HTLV1 Iranian female in Mashhad, northwestern Iran in 2012 who presented with a debilitating diarrheal illness and great weight loss. *C. belli* was detected in her stool by modified acid-fast staining and then by molecular detection. Serologic testing was negative for HIV but she showed positivity for HTLV-1 infection. Treatment with TMP/SMX led to improvement of her diarrhea but she died after one year due to malabsorption syndrome. Adequate detection of *C. belli* diarrhea in immunocompromise patients of HTLV1 in endemic area can be cured by TMP/SMX.

Introduction

Cystoisospora belli is an obligate homoxenous intracellular protozoan in phylum Apicomplexa which is located in epithelium of upper small intestine of human and causes diarrheal diseases. Since human is the only known natural host of the disease, transmission of *C. belli* oocysts seems to be confined to the anthroponotic cycle.

Immunosuppressed patients, such as those with HIV1/AIDS, as well as immunocompe-

tent individuals are frequently encountered with cystoisosporiasis; however, human cystoisosporiasis has been more commonly identified as an opportunistic infection of the gastrointestinal tract of those with low CD^{4+} lymphocyte counts (usually < 200 cells/µl) (1, 2).

Human T-lymphotropic Virus Type I or HTLV-1 is a human RNA retrovirus known to cause adult T-cell leukemia. It is a kind of lymphoma which is mostly prevalent in Japan, Middle East, Africa, the Caribbean Islands, and South America (3, 5). In the Middle East, the data about the prevalence of HTLV-1 in Iran are limited to Mashhad, Northeast of Iran, as an endemic area (3). Up to now, in a literature review, few groups have reported cystoisosporiasis in patients with HTLV-1 (6-9).

In the present report, we aimed to describe severe watery persistent diarrhea due to cystoisosporiasis in a patient with HTLV-1 in Mashhad.

Case Report

A 46-yr-old woman in 2012 living in a small village around Mashhad, Northwestern Iran with chronic diarrhea was admitted to the Emergency Ward of a hospital in Mashhad and diagnosed with acute gastroenteritis. The patient had a history of sera positive for the congenital HTLV-1 virus and HMS/TSP (HTLV-I-Associated Myelopathy/Tropical Spastic Paraparesis) paralysis of two legs from this disease in childhood. She had also a history of six months of suffering from diarrhea; the severity of her disease had dramatically increased in the previous ten days. She had bulky, fulminate, and frequently malodorous green watery diarrhea without blood (every 45 min), intermittent fever and chills, night sweating, abdominal pain, severe weight loss, malnutrition and complains of a constant feeling of pressure focused in the colon especially below the navel.

Duodenal and gastric mucosal biopsies showed chronic inflammation with no villous abnormality and active gastric mucosal atrophy. In addition, laboratory examination showed hemoglobin of 14.1 g/dL, leukocyte count of 2900/mm3 (polymorphonuclear 58%, lymphocytes 35%, monocytes 5%, and eosinophils 2%), aspartate aminotransferase of 23 U/L, alanine aminotransferase of 22 U/L, alkaline phosphatase of 361 U/L, and total bilirubin of 1 mg/dL. Hormonal and biochemical test results were normal. The patient's serum was negative for anti-HIV antibodies and HBV antibodies by ELISA.

Diarrhea was successfully treated after administration of TMP-SMX in two weeks and was followed up. However, she died after one year of treatment because of malabsorption syndrome.

Stool samples investigation

Parasitology investigation of diarrheic stool sample at the first run was reported to be negative. Two days later, however, numerous oocysts of *C. belli* were clearly and frequently seen in a second sample using the Willis flotation method in direct smear and the modified Ziehl-Neelsen staining (Fig. 1). No other parasites or pathogenic bacteria were found in the stool sample.

DNA Extraction

After microscopic examination, stained fecal smears were stored at room temperature, and DNA was then extracted from each fecal smear with a modified protocol as described previously within 2 weeks of preparation (10).

Nested-Polymerase Chain Reaction

Nested primer sets were used for amplifying fragments of the small subunit ribosomal RNA (SSU-rRNA) and the internal transcribed spacer 1 (ITS1) region of the rRNA. The outer primers were F1: 5'-CCGTTGCTCCTACCGATTGAGTG-3' and R1: 5'-GCATTTCGCTGCGTCCTTCATCG-3' with PCR amplification was performed for 5 min at 94 °C for one cycle, followed by 45 cycles using denaturation at 94 °C for 30 sec, annealing for 20 sec at 62 °C and extension

for 35 sec at 72 °C. Inner primers were F2: 5'-GATCATTCACACGTGGCCCTTG-3' and R2: 5'-GACGACGTCCAAATCCACAGAGC-3' with PCR amplification was performed for 5 min at 94 °C for one cycle, followed by 45 cycles using denaturation at 94 °C for 30 sec, annealing for 20 sec at 68 °C and extension for 35 sec at 72 °C producing an amplified product of 450 bp. The amplification products were detected by gel electrophoresis using 1.5% agarose gel in TAE buffer. DNA bands were visualized using 0.5% ethidium bromide in the presence of ultraviolet light (10).

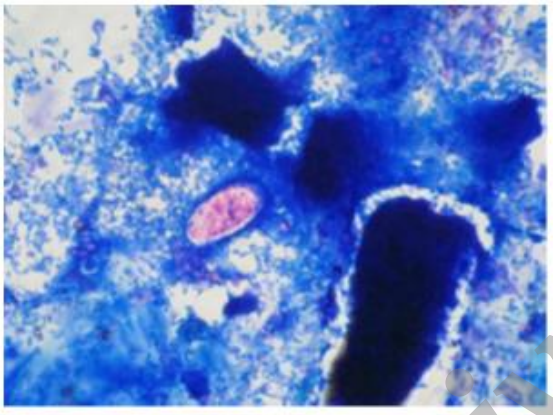

Fig. 1: *Cystoisospora belli* oocyst in a direct wet mount smear after staining with modified Ziehl-Neelsen before concentration (Original)

Gene Sequencing

For DNA sequencing, PCR products (450 bp) were purified from the agarose gel (Fig. 2), using PCR purification kit (Bioneer, Korea), and sequenced from both directions (Applied Biosystems, DNA Analyzers Sequencing, Bioneer, Korea, Sanger method), using the same primers as those used in primary PCR. ITS1, SSU- rRNA, of *Cystoisospora* sp. was analyzed and deposited in GenBank (Accession numbers: KF686747). Ninety-nine percent similarities (representative GenBank accession HM630353: 99% identity, 99% query coverage, BLAST E value 0.0) were obtained in comparison of these sequences with all available data of *C. belli* in GenBank.

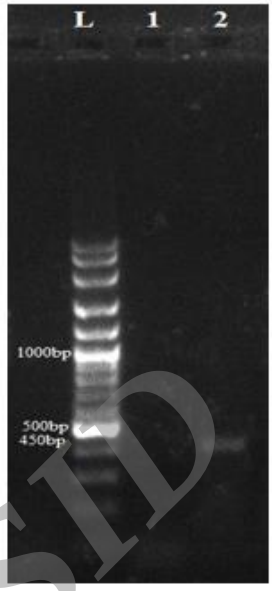

Fig. 2: Gel electrophoresis of PCR product from a *Cystoisospora belli* infected patient. L. 100-bp DNA ladder. Lane 1. Negative control. Lane 2. 450bp PCR product of *Cystoisospora belli*

Discussion

C. belli is a cosmopolitan parasite which is endemic in tropical and subtropical regions, but has been infrequently detected in stool specimens. Cystoisosporiasis is often an important opportunistic infection in HIV-infected patients, especially in developing countries (11, 12). It has also been observed in patients with concurrent Hodgkin's, non-Hodgkin's lymphoproliferative disease, acute lymphoblastic leukemia (13) and in renal and liver transplant patients (13-15), leading to diarrhea. In Iran, there are some registered cases of disorders accompanied by *C. belli* infection (16-19). However; there is no report available on the prevalence of cystoisosporiasis in Iran. In those reports, *C. belli* was usually found by direct smear and staining, but there is only one report of molecular detection of *C. belli* infection in HIV patients in Iran (20).

Hereby, we report parasitological and molecular detection of *C. belli* in other immuno-

suppressive conditions like HTLV-1. One of the endemic areas of HTLV-1 in the world is Mashhad, Northeast of Iran (3, 21). *C. belli,* as an opportunistic pathogen, can infect and cause diarrhea in the patients living this area. There are some reports of *C. belli* infection in the patients with HTLV-1. One was a report of a 65 year-old Japanese man with chronic ATLL (7). Another one was a case of a 53 yr-old Japanese man whose clinical symptoms of diarrhea were recovered after primary parasitic treatment, and 20 days after the treatment, they were relapsed (9). In both of them, *C. belli* was found in an endemic area but in an another report a 44-yr-old Sudanese- American female patient from Sudan who had been living in the United States for the previous ten years before presenting with *C. belli* infection (8). Clinical manifestations of that patient were similar to those of our patient especially weight loss which might have resulted from the combined effects of chronic diarrhea and the underlying malignancy.

In our patient, HTIV-1 caused HAM/TSP and severe diarrhea due to *C. belli* and led to weight loss as well as severe dehydration and one year after the treatment of the disease, she died.

Conclusion

Cystoisosporiasis may cause an occult infection in immunosuppressive conditions like HTLV-1. Treatment of *C. belli* oocysts in patients with acute diarrhea is very difficult. Clinically, watery acute or chronic diarrhea, with a frequency of five or six times a day, accompanied by significant weight loss is important symptoms for diagnosis of infection. TMP/SMX tablets are adequate for prophylaxis for individuals from endemic regions.

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