Disseminated Cryptosporidiosis in an Infant with Non-Hiv Pediatric Immunodeficiency: First Case Report from Oman and Literature Review

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ABSTRACT

Introduction: *Cryptosporidium* is a rare but important pathogen, especially in children with immunodeficiency. Intestinal cryptosporidiosis is well described in both immunocompetent and immunocompromised children, but respiratory and disseminated cryptosporidiosis in immunodeficient children is not often reported. **Case presentation:** We describe an Omani infant with disseminated cryptosporidiosis and failing pharmacological therapy in the context of severe combined immunodeficiency. **Conclusion:** Chronic diarrhea can be an initial symptom of immunodeficiency in the pediatric population. Awareness of cryptosporidiosis is critical to early detection and management for such patients. As antiparasitic agents are often ineffective, amelioration of immunosuppression in immunodeficient children should be a priority.

Keywords: dissemination, child, Omani, immunodeficiency, *Cryptosporidium*, diarrhea

INTRODUCTION

While the prevalence of human immunodeficiency virus (HIV) infection in Oman continues to be < 1%,[1] the prevalence of pediatric immunodeficiency (PID) is 7.0/100,000, with an estimated incidence of 5.0/100,000. These rates are higher in Oman than in Western populations, possibly reflecting a higher degree of consanguinity. Severe combined immunodeficiency (SCID) is the third most common type of PID (17.8%), following phagocytic and antibody disorders, whereas chronic diarrhea as the clinical presentation of PID is the fourth most common (10.7%) symptom, following pneumonia, deep abscesses, and Bacillus Calmette-Guérin (osis).[2] Although no local data are available, intestinal cryptosporidiosis in children with PID is probably rare.

The incidence of cryptosporidiosis in Omani children is 1.9%. *Cryptosporidium* is the third most common protozoal organism identified in stool samples in children with intestinal symptoms.[3] This parasite is increasingly recognized as an important zoonotic, food-, and waterborne enteric pathogen causing diarrheal illness in children in developing countries.[4-7] Oocysts are transmitted fecal–orally, can resist routine chlorination and ozonation of water sources,[8] and are excreted in the stool of an infected host. Although numerous *Cryptosporidium* species have been identified, humans are most frequently infected with *Cryptosporidium hominis* and *Cryptosporidium parvum*.[6] Infection is usually self-limiting or asymptomatic in immunocompetent hosts but chronic and debilitating in immunocompromised children, especially in those with profound T-cell lymphopenia and poor T-cell function.[4-10] In children with immunodeficiency, the parasites infect, and develop within the microvillus layer of small intestinal epithelial cells. Chronic infection is associated with villus atrophy, crypt hyperplasia, and secondary leucocytic infiltration in the lamina propria.[7] In addition to chronic and considerable diarrhea, growth stunting, biliary tract disease, pancreatitis, and respiratory tract disease may occur.[11] Treatment with antiprotozoal agents is usually ineffective in the context of immunodeficiency since they exhibit parasitistatic rather than parasiticidal activity.[12]

This report presents the description of the first Omani infant diagnosed with disseminated cryptosporidiosis in the context of SCID. The diagnostic and management challenges are highlighted along with a review of literature.

CASE REPORT

A 6-month-old girl was born to consanguineous parents at 37 weeks of gestation and diagnosed with interleukin-7 receptor alpha deficiency, a monogenic cause of SCID. She was introduced to oral mashed meals in the first few weeks of life using un-boiled tap water derived from local wells (a culturally acceptable practice). She was apparently well until 40 days of life when she presented with profuse watery non-bloody diarrhea with vomiting. Upon admission, fever, cough, poor growth, and severe dehydration were observed. She exhibited severe wasting and a failure to thrive (weight, height, and head circumference were all below the third percentile). Preliminary investigations revealed electrolyte imbalance with metabolic acidosis, bilateral infiltrates on chest X-ray, and hepatomegaly and gallbladder wall thickening with debris on ultrasound of the abdomen. Computed tomography of the chest revealed non-specific bilateral ground-glass opacities, potentially related to a partially treated or ongoing pneumonia. The microbiological workup included analysis of bronchoalveolar lavage (BAL) and colonic tissue culture, revealing polymicrobial infection. Virus PCR, *Mycobacterium* PCR and cultures, and *Pneumocystis* PCR were negative (Table 1). Stool examination using Ziehl–Neelsen staining was intermittently positive for *Cryptosporidium*, and colonic tissue histopathology revealed features of colitis, lymphocytic infiltrates, crypt abscesses, and the appearance of epithelial and cryptic basophilic spherical structures consistent with *Cryptosporidium* (Figure 1). In view of the microbiological workup, she was given meropenem for two weeks, voriconazole and caspofungin (sequentially) for four weeks, and paromomycin and clarithromycin (as antiparasitic agents; nitazoxanide was not available initially) for four weeks. The bacterial and fungal infections were controlled with antimicrobial therapy. Meanwhile, she was provided total parenteral nutrition (TPN), reaching a total fluid requirement of 250 mL/kg/day. However, after four weeks of pharmacological anti-*Cryptosporidium* treatment and maximum nutritional support, neither diarrhea nor weight loss had improved. Her birth weight and weight at that point in time were the same (2.71 kg). Additionally, she continued to have intermittent vomiting, wet cough, tachypnea, tachycardia, and persistent high-grade fever. Therefore,

disseminated cryptosporidiosis was suspected. Bronchoscopy and colonoscopy were repeated. Pulmonary and colonic cryptosporidiosis was confirmed by microscopy, cytology, and qualitative real-time PCR. Histopathology findings were consistent with cryptosporidiosis. Nitazoxanide (100 mg twice daily) was added, and paromomycin was discontinued with no apparent change in her clinical status for another eight weeks. Ultimately, she was sent to the hematopoietic stem cell transplantation (HSCT) center for primary cure using a haploidentical donor. Unfortunately, she did not engraft; continued to have symptoms compatible with disseminated cryptosporidiosis with lung, hepatobiliary, and colonic involvement; and ultimately died of overwhelming sepsis at the age of eight months.

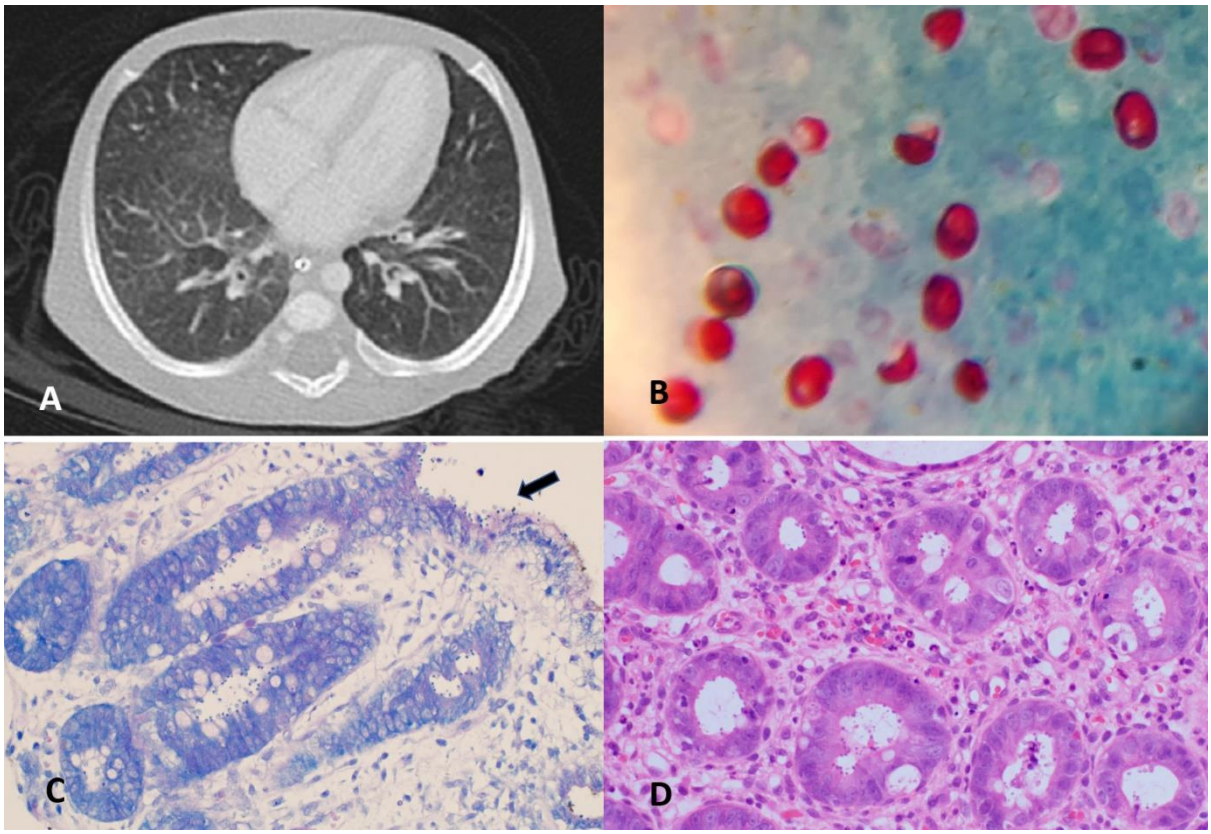

Figure 1: A: a CT scan of chest showing scattered faint ground-glass appearance related to non-specific pulmonary infection. B: cryptosporidium oocyte in acid fast stain. Oocyst appear as red oval shaped in blue/green background. C: a modified Ziehl-Neelsen stain on low power showing the cryptosporidium oocytes within the crypts and over the epithelial surface (arrow). D: hematoxylin and eosin stain of colonic tissue on high power reveal features of colitis and the presence basophilic spherical organisms within the crypts, morphologically consistent with cryptosporidium.

Table 1: biochemical, microbiological and histopathological features.

Test	Value	Normal Range	Unit	Test	Comment
Hemoglobin	8.3	10.5-13.5	g/dl	BAL culture	Pseudomonas aeruginosa.

					Candida albicans.
Platelet count	388	150-450	10*9/l	Colon tissue culture	Klebsiella pneumoniae Enterococcus faecium
White cell count	2.1	6-17.5	10*9/l	Blood culture	Enterococcus faecium
Lymphocytes	0.2	4-10.5	10*9/l	Stool viral PCR	No virus detected.
CD4+ count	2.00	600 -1200	cells/ul	Faeces culture	Negative
CRP	73.9	<5	mg/l	Cryptosporedium stain in stool	2/3 samples positive.
ALT	127	0-40	Iu/l	Respiratory viral panel	Positive for rhinovirus
AST	175	5-35	Iu/l	HIV-1 RNA viral load (blood)	Not detected.
GGT	148	10-205	Iu/l	PCR (blood, BAL) for EBV and CMV	Not detected.
Albumin	18	35-50	g/l	Adenovirus PCR (blood, BAL, stool)	Not detected.
Colonic tissue histopathology: Morphological features of immunodeficiency syndrome. Colonic cryptosporidium parvum infection. Active colitis with no granulomatous changes and no CMV or other viral inclusions.				TB culture (blood, BAL, tissue)	Not detected.
				Pneumocyctis jiroveci PCR (BAL)	Not detected.

(CRP: c-reactive protein, ALT: alanine aminotransferase, AST: aspartate transaminase, GGT: gamma-glutamyl transferase, HIV: human immunodeficiency virus, RNA: ribonucleic acid, EBV: epstein barr virus, CMV: cytomegalovirus, TB: tuberculosis).

DISCUSSION

We present the first immunocompromised Omani infant with disseminated cryptosporidiosis. This *Cryptosporidium* infection was probably acquired by ingestion of contaminated water at a very early age. Subsequent hepatobiliary disease and aspiration of oocysts to the lungs may have occurred. However, hematogenous spread could not be ruled out. Severe T-cell deficiency

contributed to the persistence and spread of the infection. Despite a high calorie/protein intake, this infant exhibited no weight gain, complicating morbidity, and resulting in mortality.

Worldwide epidemiological data on cryptosporidiosis in children with PID are limited. Aluri et al. recently studied 52 SCID patients, where 18 patients had chronic diarrhea, one of whom had cryptosporidiosis.[13] Davies et al. reported that 3 out of 42 children with PID undergoing HSCT in Northern Europe were infected with *Cryptosporidium*.[14] Surveying 34 children with PID in Warsaw, Bednarska and colleagues revealed cryptosporidiosis in two children with SCID and hyper-IgM syndrome.[15]

The prevalence of *Cryptosporidium* infection in HIV-seropositive children is quite variable. Hunter and Nichols reviewed 18 studies, identifying a mean prevalence of 32%.[11] The correlation between intestinal cryptosporidiosis and immunosuppression was studied by Legrand et al., where the median CD4 cell count was 60/mm^3 (range, 0–234) in immunocompromised individuals,[16] and Vanathy et al. claimed that a CD4 count <200 cells/mm^3 is a risk factor for severe disease and dissemination.[17]

Stool analysis was occasionally positive for *Cryptosporidium* in this patient. Fecal "ova and parasite"-positive yield may vary because of differences in oocyst shedding, stool concentration, and the availability of experienced technical personnel.[17] Meanwhile, it is crucial to involve a clinical microbiologist, employ trained staff for microscopic evaluation of at least three samples, and use molecular methods to ensure appropriate diagnostic workup and increase diagnostic sensitivity. PCR-based detection and the use of immune-fluorescence microscopy are considered the gold standard for diagnosis. A nested-PCR protocol to amplify the 18S small-subunit ribosomal RNA gene is a powerful tool to identify infection in sputum, stool, and tissue.[17] Mor et al. studied the yield of nested and restriction fragment-length polymorphism PCR in respiratory secretions in children with cryptosporidiosis. A high rate of detection was demonstrated in sputum samples as compared with saliva samples, highlighting the need of BAL fluid analysis for critically ill and non-expectorating children.[18]

Histological examination and transmission electron microscopy examination have been used to diagnose Cryptosporidium infection. Tissue analysis can reveal distinct forms of the life cycle. However, missing the infected site during biopsy and the small size of the organism can lead to false-negative results.

Despite receiving antiparasitic management from the time of diagnosis, dissemination was strongly suspected as the clinical condition failed to improve. Oral and parenteral rehydration and correction of acid–base imbalance and electrolyte disturbances are the mainstay of supportive care in intestinal cryptosporidiosis, with TPN and antidiarrheal compounds as additional supportive measures usually providing a good outcome. Antiparasitic treatment might be of modest effectiveness in immunocompetent children. However, the efficacy of such agents in children with immunodeficiency may be limited. Apparently, there is no recommended dose for antiparasitic agents in children with PID. Nitazoxanide, an antiprotozoal, and first-in-class broad-spectrum antiviral, is an FDA-approved treatment for cryptosporidiosis in immunocompetent children. However, its effectiveness and dosing regimen remain poorly delineated for immunodeficient children. Alternative treatment options include paromomycin, clofazimine, and add-on therapy with azithromycin or clarithromycin.[19] For our patient, antiparasitic treatment with paromomycin and nitazoxanide with the addition of a macrolide did neither result in control of the infection nor improve intestinal and pulmonary symptoms despite treatment for about 3 months. In a meta-analysis, Abubakar et

al. found no reduction in i) the duration of diarrhea, ii) mortality, or iii) parasitological clearance when using either nitazoxanide or paromomycin in HIV-seropositive patients.[20] Legrand et al. observed resolution of diarrhea after a mean of 5-week course of nitazoxanide and azithromycin in three children with intestinal cryptosporidiosis receiving HSCT for PID. However, their median CD4 number increased to 513 (133–615) at the end of therapy, indicating the importance of immune function restoration in addition to antimicrobial therapy.[16] Antiviral therapy in HIV-seropositive children with intestinal cryptosporidiosis can result in immune restoration and parasite eradication similar to children with PID undergoing HSCT.[21] Recently, a piperazine-based lead compound showed effective *Cryptosporidium* elimination in highly immunocompromised NSG mice.[12] This holds promise for effective pharmacological treatment while awaiting immune reconstitution in children with primary and secondary immunodeficiency statuses.

CONCLUSION

In Oman, children with suspected or confirmed PID should consume sterile water to decrease the risk of protozoal infection. Cryptosporidiosis in the context of immunodeficiency is of great concern. Therefore, high awareness and urgent action are critical, especially when the clinical condition is deteriorating. Utilization of PCR techniques and consulting expertise in microbiological diagnostics in centers managing children with immunodeficiency are crucial. Urgent reconstitution of immune function in children with PID appears conducive to a positive outcome for disseminated cryptosporidiosis when compared with pharmacological therapy alone.

CONSENT

Consent was obtained from the parents.

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