Respiratory cryptosporidiosis in two patients with HIV infection in a tertiary care hospital in Morocco

Cryptosporidiose respiratoire chez deux patients infectés par le VIH dans un hôpital de soins tertiaire au Maroc

Abstract. Respiratory cryptosporidiosis is recognized as a late-stage complication in persons with AIDS. We report two cases of respiratory cryptosporidiosis in patients with HIV infection. The first patient was a 46-year-old person with chronic diarrhea, a two-month history of low-grade fever, progressive dyspnea and productive cough. The search for acid-fast bacillus, *Pneumocystis jirovecii*, *Toxoplasma gondii* and *Cryptococcus* sp. in sputum was negative on several samples. The modified Ziehl has shown oocysts of *Cryptosporidium* sp. in induced sputum. The patient’s death occurred, due to electrolytes disorders. The second patient was a 45-year-old person hospitalized for chronic fluid diarrhea, complicated with weight loss, dry cough, dyspnea stage II and low-grade fever. The patient was HIV-positive with low CD4 count and pancytopenia. Acid-fast oocysts of *Cryptosporidium* sp. were observed in stool samples and induced sputum. The patient was treated daily with azithromycin 500 mg resulting of disappearance of gastrointestinal and respiratory disorders.

Key words: AIDS, Cryptosporidium

Cryptosporidiosis is a zoonotic disease that causes clinical symptoms in both humans and animals; it is a gastrointestinal illness caused by protozoa of the genus *Cryptosporidium*. There are many species of *Cryptosporidium* but *Cryptosporidium parvum* and *Cryptosporidium hominis* (formerly known as *C. parvum* anthroponotic genotype or genotype 1) are the most prevalent species in humans’ diseases [1]. *Cryptosporidium* infection is transmitted by the fecal-oral way; it results from the ingestion of *Cryptosporidium* oocysts through the consumption of fecally contaminated food or water or through direct person-to-person or animal-to-person contact. The oocysts are infectious immediately upon being excreted in feces [1].
Cryptosporidium causes intracellular infections, predominantly in the epithelial cells of intestine. Mild to moderate self-limited diarrhea is common in healthy persons, but patients with immune dysfunction can have prolonged diarrhea due to severe intestinal injury. Extra-intestinal involvement with Cryptosporidium has been stated in AIDS patients [2]. A few cases of pulmonary cryptosporidiosis with concomitant gastrointestinal infection have been reported in literature. We report two cases of respiratory localization of Cryptosporidium sp. in Human Immuno-deficiency Virus (HIV)-infected patients.

Case 1

A 46-year-old male was hospitalized for chronic fluid diarrhea evolving for two months and associated with a productive cough and progressive dyspnea (stage III). The diagnosis of HIV infection has been confirmed before the discovery of esophageal candidiasis by a gastroscopy performed on the occasion of uncontrollable vomiting, dysphagia to solids and retrosternal pain. The clinical examination found a patient panting at 30 cycles per minute, weight of 43 kg and dehydrated. The chest X-ray exam was normal but abdominal ultrasound showed acalculous cholecystitis. For the laboratory examination, iterative search for acid-fast bacillus (AFB) in sputum was negative. Parasitological testing of stool samples, performed by modified Ziehl-Neelsen method, showed the existence of acid-fast oocysts of Cryptosporidium sp. (figure 1). Induced sputum was negative in examination for Pneumocystis jirovecii, Toxoplasma gondii, Cryptococcus sp. and AFB. However, the modified Ziehl has shown oocysts of Cryptosporidium sp. (figure 2). Then, the diagnosis of respiratory cryptosporidiosis has been adopted. The CD4 + cell count was 70 cells/mm3 and the CD8 + cell count was 1,443 cells/mm3. While the high active anti-retroviral therapy (HAART) has been instituted, the patient was treated daily with azithromycin 500 mg for cryptosporidiosis, fluconazole 150 mg for two weeks for esophageal candidiasis, and cotrimoxazole prophylaxis for toxoplasmosis and pneumocystosis. The evolution was marked by a new hospitalization, one month later, for recurrent of gastrointestinal and respiratory symptoms. The parasitological testing of stool and sputum samples showed the persistence of oocysts of Cryptosporidium sp. The death occurred as a result of electrolytes disorder.

Case 2

Forty-five-year-old man, hospitalized for chronic fluid diarrhea, complicated with weight loss, dry cough and dyspnea with stage II, all lasting for one month with low fever (38 °C). Physical examination was unremarkable, and chest X-ray was normal. However, the patient was HIV-positive on ELISA and western blot. Cell blood count showed a pancytopenia: anemia (95 g/L), thrombocytopenia (108 G/L) and leukopenia of 1,86 G/L; the CD4 + cell count found 75 cells/mm3. Parasitological testing of stool samples showed the existence of Cryptosporidium sp. oocysts and Blastocystis hominis. Iterative AFB examinations in sputum were negative. However, the modified Ziehl showed oocysts of Cryptosporidium spin sputum (figure 3). The diagnosis of respiratory cryptosporidiosis was established. The patient was treated with azithromycin (500 mg a day) for cryptosporidiosis and cotrimoxazole prophylaxis for toxoplasmosis and pneumocystosis. The HAART in this case was a combination of two nucleoside reverse
Cryptosporidiosis is a gastrointestinal protozoan infection is commonly diagnosed in immunocompromised patients, essentially in developing countries. Therefore, the rapid dissemination of HIV has increased considerably the incidence and the prevalence of Cryptosporidiosis. Cryptosporidium sp. is responsible for potentially severe opportunistic infections with a morbidity rate that’s directly proportional to the depth of immunodepression. Its frequency ranged from 14 to 24% [3].

In Morocco, the prevalence of AIDS seems to be underestimated with 20,000 HIV-seropositive patients (less than 1% of the population). Unfortunately, no data are available concerning the incidence of cryptosporidiosis in this population [4].

Infection with Cryptosporidium sp. results in a wide range of manifestations, from asymptomatic infections to severe, life-threatening illness. The mean incubation period is seven days (range from two to 10 days). Diarrhea is the most frequent symptom, and can be associated with dehydration, weight loss, abdominal pain, fever, nausea and vomiting. In immunocompetent persons, symptoms are usually short-lived (one to two weeks). Conversely, they can be chronic and more severe in immunocompromised patients, especially those with CD4+ counts of less than 200 cells/mm³, as a result of the autoinfestation [1, 5].

While the small intestine is the most commonly affected site, symptomatic Cryptosporidium infections have also been reported in other organs including hepatic ducts, lungs and possibly conjunctiva [6, 7]. These extra-intestinal localizations have been rarely reported. However, a few reports of pulmonary cryptosporidiosis in HIV/AIDS cases have been mentioned in literature [8, 9]. We believe that the frequency of respiratory localizations of Cryptosporidium sp. remains underestimated. Moreover, the pathogenesis of this infection remains unknown but it precedes usually the fatal issue of associated opportunistic infections [10, 11]. Pulmonary cryptosporidiosis is observed in patients with profound immunosuppression affecting mostly cellular immunity. Therefore, a strong association between cryptosporidiosis and depleted CD4+ cell count has been established in previous reports [12, 13]. In our reports, both of cases had presented CD4+ of less than 100 cells/mm³. Respiratory cryptosporidiosis involves cough, dyspnea, low fever and abnormal chest X-ray that shows an interstitial pneumopathy [14]. Both of our patients had a diagnosis of simultaneous intestinal and pulmonary cryptosporidiosis. The first patient has presented a disseminated cryptosporidiosis with possible localization in the biliary tract; unfortunately, because of the death, no investigation was undertaken to prove it.

Acid-fast staining methods are the most frequently used in clinical laboratories for the diagnosis of cryptosporidiosis. The modified Ziehl-Neelsen method shows 5 to 8 microns acid-fact oocysts, rounded, colored in fuchsia pink, with a thick wall and granular contents [14, 15]. For greatest sensitivity and specificity, immunofluorescence microscopy is the method of choice, followed closely by enzyme immunoassays. Molecular methods are a research tool [16]. Cryptosporidium sp. is not the only cause of respiratory disorders observed in HIV infected patients; co-infections with cytomegalovirus (CMV), Mycobacterium and P. jirovecii are very common [17]. In our patients, there was no evidence of P. jirovecii, T. gondii or AFB researched by standard methods. Our technical platform did not allow eliminating other causes of co-infections, particularly viral infections. Therefore, we attribute the respiratory disorders to Cryptosporidium sp. alone, which may explain the lack of response to treatment by azithromycin in the first patient. Among the most commonly used treatments against cryptosporidiosis paromomycin, and azithromycin are partially effective. For nitazoxanide (NTZ), effectiveness was demonstrated in vitro and in vivo using several animal models and finally in clinical trials. It significantly shortened the duration of diarrhea and decreased mortality in adults and in malnourished children. NTZ is not effective without an appropriate immune response. In AIDS patients, combination therapy restoring immunity along with antimicrobial treatment of Cryptosporidium sp. infection is necessary. Investigations are now focused on many new potential treatments such as target molecular-based
immunotherapy, probiotic bacteria even that can’t totally eradicate \textit{C. parvum} and new synthetic isoflavone derivatives that demonstrated excellent activity against \textit{C. parvum} \textit{in vitro} and in a gerbil model of infection. Newly synthesized nitro- or non nitro-thiazolide compounds, derived from NTZ, have been recently shown to be at least as effective as NTZ against \textit{C. parvum} \textit{in vitro} development and are promising new therapeutic agents \cite{18}. Despite the two patients received the same therapy regimen (based on azithromycin and HAART therapy), the first patient died with electrolyte disorders and the second shows a total recovery. Death in the first case might have been due to the existence of other under-diagnosed opportunistic infections (candidiasis) as well as the existence of disseminated cryptosporidiosis (acalculous cholecystitis). We emphasize that there was no other anti-cryptosporidium alternative treatment available in our hospital.

\textbf{Conclusion}

Early identification of the etiological agent of respiratory disorders in patients with AIDS is very important as it can help in the institution of appropriate therapy and the reduction of morbidity and mortality in these patients. Microbiology laboratories should be alerted to the possibility of \textit{Cryptosporidium} sp. oocysts presence in respiratory specimens from patients with advanced HIV/AIDS disease and pulmonary involvement.

\textbf{Conflicts of interest:} none.

\textbf{References}


