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Short Communication

CRYPTOSPORIDIOSIS: AN EMERGING ENTERIC DISEASE IN HIV-INFECTED PATIENTS, BARAK VALLEY, ASSAM, INDIA

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ABSTRACT

Objective: Enteric parasites are a major cause of diarrhoea in HIV-infected people. The present study was undertaken to detect enteric parasites in HIV-infected patients with diarrhoea at different levels of immunity.

Methods: The study was carried out at the ART centre Silchar Medical College and Hospital, Assam, India, between March 2013 and March 2014 among consecutively enrolled 127 HIV-infected patients presenting with diarrhoea. Stool samples were collected and examined for enteric parasites by microscopy and special staining methods. CD4 cell counts were estimated using the rapid serological tests using Combs Aids, Pareekshak® HIV ½ Triline card test and SD Bioline anti-HIV ½ test kits as per protocol provided by the supplier.

Results: A total of 127 stool samples was collected from the HIV patients attending the antiretroviral therapy (ART) center at Silchar Medical College Hospital in Assam and were isolated for *Cryptosporidium* oocysts during the one year period from March 2013 and March 2014. The stool samples were examined by the help of modified Ziehl-Neelsen technique. It was seen that out of 127 samples, 25 were *Cryptosporidium* oocysts positive (19.68%), which is the higher than the prevalence range reported from the Southern Assam of India.

Conclusion: *Cryptosporidium* infections were detected in 19. 68 percent HIV-infected patients and low CD4 counts were significantly associated with infection. Detection of aetiologic pathogens might help clinicians decide appropriate management strategies.

Keywords: Diarrhoea, HIV, *Cryptosporidium* spp

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Cryptosporidium spp. causes severe, life-threatening diarrhoea in untreated HIV-infected patients. Until the advent of highly active anti-retroviral therapy (HAART), this was a relatively common opportunistic infection even in developed countries [1, 4]. In India, there have been reports from the mid-1990s on the prevalence of cryptosporidiosis from different parts of the country, ranging from 8.5 [5] to 81% [6] with a very high incidence reported from the northeastern states [6, 7].

Cryptosporidiosis is a parasitic disease caused by the *Cryptosporidium*, a protozoan parasite belonging to the phylum Apicomplexa. *Cryptosporidium* was discovered by Tyzzer in 1907 from the gastric glands of infected mice, and it gradually emerged as an increasingly recognized public health threat [8, 9]. It has been identified in watery diarrhoea in patients with HIV and other immune-compromised patients and in large human outbreaks of diarrhoea in both developed and developing parts around the world [10, 11]. It affects the intestines of mammals. It spreads through the fecal-oral route and contaminated water. In immune-compromised persons, such as AIDS patients, the symptoms are severe and often fatal [9, 11].

The emergence and pandemic spread of AIDS constitute the greatest challenge to public health in modern times. The first case of AIDS in India reported in 1986 from Chennai. India is now the country with the second largest population of HIV-infected individuals [12].

Cryptosporidium is a genus of protozoan parasites with species that infect fish, amphibians, reptiles, birds and mammals. In HIV-positive patients suffering from diarrhoea it is the most commonly isolated parasite. Oocysts are transmitted from an infected host to susceptible hosts. Transmission can be human-to-human, animal-to-animal, human-to-animal, and animal-to-human, waterborne, foodborne and possibly airborne. In the case of HIV-positive patients, generally, CD4 T cell numbers or function decreases, thereby increasing the severity of the infection. Infections can become chronic and life is threatening with frequent voluminous watery stools leading to dehydration [13].

The objective of this study is to examine the prevalence of *Cryptosporidium* spp. occurring in hospital-based patients with diarrhoeal symptoms during the period of one year from March 2013 to March 2014.

The study was approved by the Ethical Committee of the institution (Ref no. GCC/9440). All study participants had given written consent before enrollment into the study. Parents/guardians provided consent on behalf of all infant participants. Fecal samples were collected from a total of 127 HIV-positive patients attending the ART center of the Silchar Medical College and Hospital, Assam. *Cryptosporidium* oocysts were identified by the modified Zeihl-Neelsen technique [14]. The *Cryptosporidium*-positive samples were kept on-20 °C to be used for DNA extraction.

Isolation of *Cryptosporidium* species was carried out by modified Ziehl-Neelsen staining technique according to Potters and Esbroeck (2010) with slight modifications as given below:

Preparation of smear for staining involved applying a small sample to the center of a carefully cleaned glass slide. The smear was allowed to dry completely and then the smear was fixed with methanol. The smear was heat fixed, and air dried at 80 °C for 15 min. A slide was placed on an air dried and heat fixed smear on a suitable staining device; then the slide was flooded with Kinyoun's carbolfuchsin reagent and allowed to stain for 15 min. The slide was washed using a gentle and indirect stream of tap water until no colour appeared in the effluent. It was re-decolorized with acid alcohol for 60 seconds or until no more red colour ran from the smear. Precaution was taken with decolorize until the fluid washing off the slide ran clear. The slide was then rinsed with tap water. The slide was then flooded with counterstain methylene blue for 5 min. The slide was again rinsed with tap water and dried for examination

under the phase contrast microscope (Olympus CX-31, Japan) under 100x magnification (oil immersion) [14].

Regarding sex distribution among the participants, there were 11 (8.66%) females and 116 (91.33%) males in HIV+ group. The distribution of age in HIV+ patients as shown in fig. 1. Out of the total number of 127 HIV+ patients, 19.68% (25 cases) were infected with *Cryptosporidium* sp. In addition, the frequency of infection was observed using CD4 counting. In a group number<200 CD4, 15 patients (11.81%) were infected, in the group with CD4 counts 200-300, 8 patients (6.2%) were infected and in the group with CD4 counting>300 two patient (1.57%) had *Cryptosporidium* spp. Also, the patients for the study were divided into 5 age groups. Their number and out of 25 people infected with *Cryptosporidium* who were studied, 13 people were suffering from chronic diarrhea (table 1). The results also show that the lower CD4 counts<200 had more possibilities of *Cryptosporidium* infection and an increase in CD4 counts and consequently, an increase in immune system resistance; the rate of infection decreases (fig. 2).

In this study, the most common parasites seen in patients with diarrhoea were *Cryptosporidium* sp. (19. 68 %) having both round and oval in shapes with variable size range (3-6 µm), which appeared pink against a light-blue background (fig. 3).

Table 1: Comparison of CD4 counting with *Cryptosporidium* infection with chronic diarrhoea

S. No.	CD4 countsc CD4 counts	Diarrhoea	*Cryptosporidium* infection
1	350	+	+
2	154	+	+
3	134	+	+
4	182	+	+
5	255	+	+
6	120	+	+
7	167	+	+
8	136	+	+
9	281	+	+
10	221	+	+
11	167	+	+
12	283	+	+
13	117	+	+
14	189	+	+
15	209	+	+

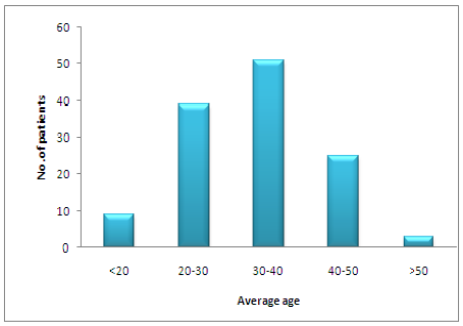

Fig. 1: Comparison age distribution with infected people in HIV+patients admitted to the ART centre silchar medical college and hospital, assam 2013-2014

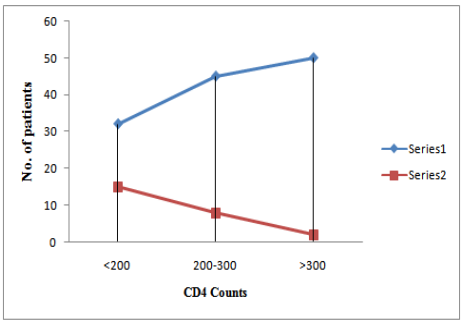

Fig. 2: Comparison infected people with CD4 counts *Cryptosporidium* in HIV+Patients admitted to the ART center silchar medical college and hospital, assam 2013-2014

Series 1: Total no of sample

Series 2: *Cryptosporidium* infection

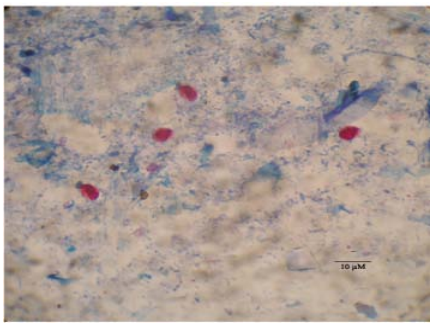

Fig. 3: *Cryptosporidium* oocysts were seen under phase contrast microscope (Olympus CX-31, Japan)

Cryptosporidium infections are the commonest and major cause of morbidity and mortality in HIV-positive cases in the worldwide. These organisms usually cause a self-limiting illness in immune-compromised patients; they can cause life-threatening, severe diarrhea [15]. HIV infection is a major threat to public health in India. It would be advisable to recognize that cryptosporidiosis can present with just chronic weight loss. Additionally, laboratory testing for *Cryptosporidium* in HIV-infected patients is highly recommended in order to have a better understanding of the epidemiology and management [16]. Modified Z-N staining method was used for identification of the *Cryptosporidium* oocysts in the present study; the study was conducted to compare the positivity of acid-fast staining technique in HIV positive diarrheal stool samples (n=127). *Cryptosporidium* infection was detected in 25/127 (19.68%) of the diarrheal stool samples from HIV-positive patients in the present study, while the same prevalence rate of *Cryptosporidium* infection was found Gupta *et al.* [17] and Amatya *et al.* [18].

Symptomatic cryptosporidiosis was first noted in turkeys in 1955 [19]. During the 1970s, *Cryptosporidium* infections were reported to cause neonatal diarrhoea in calves. The first human cases of cryptosporidiosis in humans were recorded in the 1970s, one with a young girl with enterocolitis [20]. The other records were in AIDS

patient with the spread of AIDS in the 1980s [21] and 1990s more and more cases of cryptosporidiosis were diagnosed [22]. Apart from the acute symptoms, there are potential long-term consequences of *Cryptosporidium* infections. Infection at a young age can lead to impaired development, growth and possible long-term cognitive deficits, especially among children in the developing world [23].

It must be noted that these studies can be difficult to conduct because of the stigmatizing nature of the illness. During the course of this study, many problems were encountered because patients did not return for scheduled visits to the hospital and when home visits were made, it was discovered that patients had moved or provided incorrect addresses. Such problems have also been encountered by other investigators, who have shown that approximately 70 percent of individual's returns with the results of HIV-related tests, and less than 50 percent participated in further studies [24, 25].

The modified Z-N staining technique was less sensitive for the detection of *Cryptosporidium* in the study population; however, it has the advantage of being the only technique that only indicates active infections, unlike the ELISA and PCR techniques which may not distinguish between active and non-active infections. Moreover, less expertise the Z-N staining technique, although the use of this technique means that several cases of cryptosporidiosis will go undiagnosed as it less sensitive.

The majority of patients suffering from cryptosporidiosis were immune-compromised. Modified acid-fast staining is an economical and easily applicable method in the detection of *Cryptosporidium* oocysts in faecal samples. This test should be made available routinely as a screening tool of diarrhoeal stool samples. It is not considered at risk to others and that no specific treatment is available for it. It may be rare because it is rarely investigated. It is usually self-limiting, but can be chronic and life threatening in immune-compromised patients. The diagnosis of *Cryptosporidium* is not difficult and can be achieved by simple conventional staining methods like to modified Z-N staining. However, depending on the facilities available, Z-N staining is easy to use in the detection of *Cryptosporidium* oocysts. Routine testing of stool will help to explore the severity of the infection and help to reduce morbidity and mortality associated with *Cryptosporidium* infection.

CONFLICT OF INTERESTS

Declared none

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