



RESEARCH ARTICLE

DETECTION OF CRYPTOSPORIDIUM SPECIES, ISOSPORA SPECIES NAD CYCLOSPORA SPECIES IN HIV/AIDS POSITIVE PATIENTS WITH DIARRHOEA IN A TERTIARY CARE CENTRE

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ARTICLE INFO

Article History:

Received 22nd April, 2016
Received in revised form
10th May, 2016
Accepted 06th June, 2016
Published online 31st July, 2016

Key words:

HIV and AIDS patients,
Diarrhoea, Modified acid fast stain,
Rapid saffranin method.

ABSTRACT

Intestinal coccidian infection is a major health problem in a developing country like India, having the largest number of people living with HIV in the world. This study includes 80 stool samples from AIDS patients with diarrhoea attending St. John medical college and hospital, Bangalore. *Cryptosporidium* species and *Isospora* species oocysts were detected using modified acid fast stain and rapid saffranin method. This was done in stool samples before and after concentration. *Cryptosporidium* species oocyst (2.5%) was detected in the stool samples stain with modified acid fast stain before stool concentration. An additional *Cryptosporidium* species oocyst (18.75%) was detected following stool concentration. *Isospora* species oocyst (11.25%) was detected following formalin ether concentration technique. Of the *Cryptosporidium* oocyst (3.7%) detected by the modified acid fast stain was also seen using the rapid saffranin method following stool concentration method. *Cyclospora* oocysts were not detected. Both modified acid fast stain and rapid saffranin method are useful in demonstrating oocysts of *Cryptosporidium* species and *Isospora* species. Stool concentration improved detection of oocysts.

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Citation: Onila Nongmaithem, 2016. "Detection of cryptosporidium species, Isospora species Nad cyclospora species in HIV/AIDS positive patients with diarrhoea in a tertiary care centre", *International Journal of Current Research*, 8, (07), 35259-35261.

INTRODUCTION

India has the distinction of having the largest number of people living with HIV in the world and the adult prevalence rate of 0.91%, is increasing (Banerjee *et al.*, 2005). Intestinal coccidian infections including *Cryptosporidium*, *Cyclospora* and *Microsporidia* are increasingly becoming prevalent in acquired immunodeficiency syndrome (AIDS) patients (<http://www.nacoonline.org>). In immunocompromised patients, the intestinal opportunistic parasites probably play a major role in causing chronic diarrhoea accompanied by weight loss (Agarwal, 1998). *Cryptosporidiosis* remains an important cause of diarrhoea in the immunocompromised due to the lack of effective therapy. *Cryptosporidiosis* in HIV infected patients has been widely reported in India (Muttusamy *et al.*, 2004). Failure to diagnose cryptosporidiosis early may interfere with therapeutical procedures, leading to high morbidity and mortality (Silva *et al.*, 2003). Because many patients with AIDS are being seen in India, the accurate identification of these opportunistic enteric parasites will become more important for diagnosis and management of these patients (Talib, 1998).

It is necessary to document the public health significance of this infection in immunocompromised hosts in order to formulate more rational approaches to the control of these diseases, especially in the developing countries like India (Rao Ajjampur *et al.*, 2007). The present study was under taken to study the prevalence of opportunistic parasites which causes diarrhoea and their association with their status in HIV/AIDS patients in St. John Hospital, Bangalore, India.

MATERIALS AND METHODS

This study was undertaken for the detection of *Cryptosporidium species*, *Isospora species* and *Cyclospora species* among HIV/AIDS positive patients with diarrhoea who attended St. John Medical College and Hospital, Bangalore, India. The samples from 80 HIV positive cases with diarrhoea were collected from December 2011 to January 2013. These patients had already been tested for HIV at an ICTC centre as per strategy 3 of the National AIDS Control Organisation to establish the diagnosis of HIV. Stool examination: Stool specimen were collected after taking an oral consent from the patients according to the standard procedure of the WHO and they were examined microscopically by following the direct and the formalin-ether concentration method. The stool samples were collected in labelled, leak proof, clean and sterile

plastic container and they were then transported to the laboratory within 3 hours of their collection. All specimens were collected prior to radiological studies using barium or the administration of bismuth, minerals oil and anti-diarrhoeal medication that could interfere with the detection and identification of intestinal parasites.

The faecal specimens were properly labelled with the patient name, hospital number, date and the time of collection. The stool specimens were not kept at room temperature for longer time. The specimen should never be incubated at 37°C or frozen at 0°C. These specimens can be kept in refrigerator at 4°C. An episode of diarrhoea was classified as acute if it lasted for less than a month and provided the patient is diarrhoea free in the preceding months. Episode was defined as chronic when chronic when diarrhoea lasted more than a month, or was intermitted and recurrent over a period of at least 2 months with diarrhoeal symptoms for at least half this time. Then the stool samples were examined through a direct observation in saline (0.85% NaCl). Lugol's iodine was used for the demonstration of the intestinal nuclear structure of the parasites. The smears of the direct and the concentrated specimens were examined by modified acid fast and rapin saffranin method for *C. parvum*, *I. belli* and *Cyclospora*.

RESULTS

The present study showed that out of 80 AIDS diarrhoeal stool sample, the prevalence of *Cryptosporidium* species (21.2%) oocyst was the commonest parasite, followed by *Isospora* species oocyst (11.2%). Other intestinal parasite found were *Tricuris trichura* egg (12.5%), *Giardia* cyst (6.2%) and *Strongyloides* larva (2.5%) (Table 1).

Table 1. Comparison of microscopic examination before and after concentration (Formalin-ethyl Acetate)

Microscopic method	Before concentration	After concentration
Modified acid stain	<i>Cryptosporidium</i> 2.5%	<i>Cryptosporidium</i> (18.75%), <i>Isospora</i> (11.25%), <i>Cryptosporidium</i> (3.7%)
Rapid Saffranin	nil	<i>Giardia</i> cyst (3.75%)
Iodine mount	<i>Giardia</i> cyst (2.5%)	<i>Trichuris-trichura</i> egg (8.7%)
Saline mount	<i>Trichuris-trichura</i> egg (3.75.2%)	<i>Strongyloides</i> larva (1.25%)
	<i>Strongyloides</i> larva (1.25%)	<i>Ascaris</i> egg (1.25%)
	<i>Ascaris</i> egg (1.25%)	nil

Table 2. Age and sex distribution of cases

	Total male	Percentage	Total female	Percentage	Total	Percentage
30-40	18	36%	12	24%	30	60%
41-50	12	24%	6	12%	18	36%
51-60	2	4%	0	0	2	4%

DISCUSSION

In the present study, there is a male predominance as similar in studies conducted by S.V. Kulkari, S.Gupta as well in other studies (8,9). Weight loss (70%), fever (48%) was the most common complained. Most of the symptomatic cases presented with chronic diarrhoea, which is similar to the study by S.Satheesh. (10) In our study, *Crptosporidium* (18.7%) and

Isospora (11.25%) was detected. No *Cyclospora* was detected in the present study similar with some other studies. However, in the study conducted by Kumar *et al*, they detected *Cyclospora*. (11). Out of the 80 stool samples, 2.5% of *Cryptosporidium* oocyst was detected before concentration and 18.7% after concentration and staining by modified acid fast. *Isospora* oocyst (11.25%) were detected only after concentration. Using rapin saffranin method, 3 (3.7%) of the above *Cryptosporidium* oocyst was detected. Modified acid fast stain may a better method to detect *Cryptosporidium* compared to rapid saffranin method. A larger number of samples need to be studied to determine if this observation is true.

Conclusion

Cryptosporidium species and *Isospora* species are associated in AIDS patients. The oocysts can be detected using modified acid fast and rapid saffranin method. Stool concentration enhances detection of *Cryptosporidium* and *Isospora* oocysts. Detection of *Cryptosporidium* and *Isospora* species will help in proper management of patients.

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