

Comparative Study of Modified Acid Fast Stain and Rapid Saffranin Method for the Better Detection of *Cryptosporidium*, *Isospora* and *Cyclospora* Oocysts in HIV Diarrhoeal Samples

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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. Objectives: 1. To detect opportunistic parasites in HIV/AIDS positive patients with diarrhoea. 2. To compare the performances of modified acid fast and rapid saffranin method for the detection of *Cryptosporidium* species, *Isospora* species and *Cyclospora* species in HIV/ AIDS patients with diarrhoea. 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.

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

1. 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 [1]. Intestinal coccidian infections including *Cryptosporidium*, *Cyclospora* and *Microsporidia* are increasingly becoming prevalent in acquired immunodeficiency syndrome (AIDS) patients [2]. In immunocompromised patients, the intestinal opportunistic parasites probably play a major role in causing chronic diarrhoea accompanied by weight loss [3]. *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 [4]. A conventional method for identification includes wet mount, trichrome stain, merifluor

stain & auramine–rhodamine stains. [12] These methods are time consuming and trichome stains results cannot give the confirmation for *Cryptosporidium*, as all oocysts will appear unstained [12]. There is also a lack of appreciation of the importance of this opportunistic parasite in immunocompromised host because of the low prevalence. Therefore failure to diagnose and difficulty in testing of these infections early may interfere with therapeutic procedures, leading to high morbidity and mortality. [5]

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 [6]. 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 [7]. 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.

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2. 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*.

3. 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%).

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. In the similar studies done by S.V Kulkarani & A. Singh also reported the same whereas in studies done by S. Satheesh, vomiting & abdominal pain was predominant complaint [8]. Most of the symptomatic cases presented with chronic diarrhoea, which is similar to the study by S. Satheesh [10]. However cases presented with acute diarrhoea in the study conducted by S.Gupta and S.S Rao Ajjampur [7].

In our study, *Cryptosporidium* (18.7%) and *Isospora* (11.25%) were detected. No *Cyclospora* was detected in the present study similar with some other studies done by S.Satheesh et al & SS Rao [8, 7]. However, in the study conducted by Kumar et al & S.V Kulkarni et al, they detected *Cyclospora*. [11].

Table 1. Comparision 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%),
Rapid Saffranin	nil	<i>Cryptosporidium</i> (3.7)
Iodine mount	<i>Giardia</i> cyst (2.5%)	<i>Giardia</i> cyst (3.75%)
Saline mount	<i>Trichuris-trichura</i> egg (3.75.2%) <i>Strongyloides</i> larva (1.25%) <i>Ascaris</i> egg (1.25%)	<i>Trichuris-trichura</i> egg (8.7%) <i>Strongyloides</i> larva (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%

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%) was detected only after concentration followed by modified acid fast stain. Using rapid saffranin method, 3 (3.7%) of the above *Cryptosporidium* oocyst was detected. This result has agreements with other similar studies done by S.H Talib, S.Gupta where most of the organism yields better results in Modified acid fast stain. Modified acid fast stain may a better method to detect *Cryptosporidium* compared to rapid saffranin method. Stool concentration evidently improves detection of *Cryptosporidium* species and *Isospora* species as evidenced by other studies [13, 9].

Other intestinal parasites present in this study were *Giardia*, *Trichuris trichura*, *Strongyloides* larva and *Ascaris* egg. *Ascaris* egg was identified before concentration. In other studies, they have reported *Giardia*, *Ascaris lumbricoides*, *E. histolytica*. [13, 14]. *Trichuris trichura* egg was also detected in one *Cryptosporidium* species positive stool sample.

The study was limited as only single stool samples were examined and lack of molecular method which could increase the detection of coccidian parasites more, also larger number of samples need to be studied to determine if this observation is true.

5. Conclusions

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.

REFERENCES

- [1] Banerjee I, Primose B, Roy S, Kang G. Enteric parasites in patients with diarrhoea presenting to a tertiary care hospital: Comparison of Human immunodeficiency virus infected and uninfected individuals. J Assoc Physicians, India 2005; 53: 492-4.
- [2] HIV/AIDS epidemiological Surveillance and Estimation report Available from: <http://www.nacoonline.org>.
- [3] Agarwal A, Ningthouja S, Sharma D, Mohen Y, Singh NB. *Cryptosporidium* and HIV, J Indian Med Assoc 1998; 96: 276-7.
- [4] Muttusamy D, Rao SS, Ramani S, Monica B, Banerji I, Abraham OC, et al. M ultilocus genotyping of *Cryptosporidium* species isolates from human immunodeficiency virus-infected individuals in South India J ClinMicrobiol 2006; 44:632-4.
- [5] Silva CV, Ferreira MS, Goncalves- PiresMdo R, Costa-Cruz JM. Detection of cryptosporidium-specific coproanigen in human immunodeficiency virus/acquired immunodeficiency syndrome patients by using a commercially available Immunodeficiencyassay. MemInst Oswaldo Cruz 2003; 98: 1097-9.
- [6] SH Talib, Jeet Singh. A study on opportunistic enteric parasites in 80 HIV seropositive patients. Indian J.Pathol Microbiol, 41(1):31-37, 1998.
- [7] SS. Rao Ajampur, J.R. Asirvatham, Dheepa Muthuswamy, B.P. Gladstone, O.C. Abraham Dilip Mathai, Honorine Ward, Christine Wanke & Gagandeep Kang. Clinical features & risk factors associated with cryptosporidiosis in HIV infected adults in India. Indian J Med Res: 2007:126:553-557.
- [8] Kulkarni S.V, Kairon R, Sane S.S, Padmavan P.S, Kale V.A, Thakar M.R and Risbad A.R; Opportunistic parasitic infection in HIV/AIDS patients presenting with diarrhoea by the level of immunosuppression. Indian J Med Res 130. July 2009. pp63-66.
- [9] Gupta S, Narang S, Nunavath V, Singh S. Chronic diarrhoea in HIV patients: Prevalence of coccidian parasites. Indian J Med Microbiol 2008:26:172-5.
- [10] S. Satheesh Kumar, S. Ananthan and P. Saravanam: Role of coccidian parasites in causation of diarrhoea in HIV infected in Chennai. Dept of Microbiology, Dr. ALM Post Graduate Institute of Basic Medical Sciences. Chennai, India. Indian J Med Res 116, September 2002, pp 85-89.
- [11] Kumar SS, Ananthan S, Lakshmi P. Intestinal parasitic infection in HIV infected patients with diarrhoea in Chennai. Indian J Med Microbiol 2002:20(2):88-91.
- [12] Kehl S.C, Cicirello H, Peter H: Comparison of four different methods for detection of *Cryptosporidium* species. J Clin Micro, 1995:416-418.
- [13] Dwivedi KK, Prasad G, Saini S, Mahajan S, Lal S, Baveja UK. Enteric opportunistic parasite among HIV infected individuals: associated risk factors and immune status. Jpn Infect Dis 2007:60:76:81.
- [14] Beena U, Kashyap B & Bhalla P: Enteric Pathogens in HIV/AIDS from a Tertiary Care Hospital. Indian J Community Med.2009 July: 34(3):237-242.