Original Research Article

Detection of Oocysts of cryptosporidum in stool samples at a tertiary care hospital

Kumudini T.S[1], Narayan Shrihari[2*]

[1]Assistant Professor, Department of Microbiology, Vijayanagar Institute of Medical Sciences, VIMS, Ballari, Karnataka, India

[2]Associate Professor, Department of Microbiology, Vijayanagar Institute of Medical Sciences, VIMS, Ballari, Karnataka, India

Received: 05-06-2021 / Revised: 12-07-2021 / Accepted: 10-09-2021

Abstract

Background: Diarrhoea is a common complication of infection with HIV/AIDS leading to weight loss. It occurs in almost 90% of the immunocompromised and HIV patients. Cryptosporidium parvum is a major cause of diarrhoea in developing countries; mainly affecting HIV infected individuals with low CD4 counts. The infection is self limiting in immunocompetent hosts but can be severe and persist in the immunocompromised individuals. **Objective:** To detect oocysts of Cryptosporidium parvum by modified Ziehl-Neelsen staining in stool samples of patients attending our Hospital. **Material and Methods:** Stool samples from forty patients with diarrhoea were examined for oocysts by modified Ziehl-Neelsen (ZN) staining. **Results:** Of the 40 stool samples, modified ZN staining detected 19 (47.5%) stool samples positive for oocysts of Cryptosporidium parvum. **Conclusion:** There is a high prevalence of cryptosporidiosis in immunocompromised patients and simple method modified ZN staining can detect oocyst in stool sample.

Key-words: Oocysts and Cryptosporidium parvum

Introduction

Infections associated with the HIV are being increasingly reported from South East Asia and a large number of patients either undiagnosed or present with multiple infections[1]. The etiology spectrums of enteric pathogens is broad including bacteria, parasites, fungi and viruses. Among HIV/AIDS patients in developing countries as many as 95% may have bacteria[1]. In a review of HIV related opportunistic infections in Northern India, chronic diarrhoea was the second most common[1]. Parasitic infections with Isospora belli, Entamoeba histolytica and Cryptosporidium parvum have been reported as being the most frequently identified organisms in India[2]. Cryptosporidium parvum is a small parasite measuring about 3-5µm in diameter. The life cycle of Cryptosporidium parvum is completed within the small intestine and colon of the host with the developing stages associated with the luminal surface of the intestinal epithelial cells where it remains intracellular but extracytoplasmic[3].

Transmission occurs by ingestion of contaminated food or water or contact with infected animals (beef, cattle, sheep, pig and dog). Cryptosporidium parvum ingested as an oocyst, undergoes excystation and sporozoites parasitizes the host. The oocysts are highly resistant to common household disinfectants and survive for long periods in the environment[3]. Identification of oocysts in stool samples has been described, modified Ziehl-Neelsen (ZN) staining (1% sulphuric acid must be used)[4,5].

*Correspondence

Dr. Narayan Shrihari

Associate Professor, Department of Microbiology, Vijayanagar Institute of Medical Sciences, VIMS, Ballari, Karnataka, India.

E-mail: shriharimicro@gmail.com

The purpose of this study was to find prevalence of cryptosporidiosis in HIV/AIDS and immunocompromised individuals, creating awareness among the population and to highlight the importance of detection of oocysts in routine stool examination.

Material and methods

Study design

A prospective study

Study location

Department of Microbiology, Vijayanagar Institute of Medical Sciences (VIMS), Ballari

Study Duration

Six months (May 2019 to October 2019).

A prospective study was carried out in the Department of Microbiology for a period of six months (May 2019 to October 2019). Forty stool samples from immunocompromised patients with symptoms of diarrhoea were included in our study. The patients were provided a wide mouthed, clean, dry, properly labeled plastic container for collection of stool samples. Five grams of formed or about 10 ml of unformed stool was collected and samples were examined within one to two hours of collection. The smears were prepared from stool samples and stained by modified Ziehl-Neelsen staining[4,5]. The stained smears were examined under oil for oocysts of Cryptosporidium parvum. [Figure No: 1].

Results

Out of the 40 cases stool samples were studied, 39 cases (97.50%) presented with diarrhoea where as one case (2.50%) did not have diarrhoea. The number of males presenting with diarrhoea was more than that of the females [Table No: 1].The prevalence of Cryptosporidium parvum in our study is 47.50% (19/40) [Table No: 2].The number of stool samples considered positive in our study was 19 out of 40 stool samples.

Table 1: Gender and Diarrhoea wise distribution of cases (n=40)

Gender	With diarrhoea	Without diarrhoea	Total
Male	29	01	30
Female	10	0	10

Table 2: Cryptosporidium parvum Oocysts positive cases

No. Stool tested	No. Positive	Percentage (%)
40	19	47.5

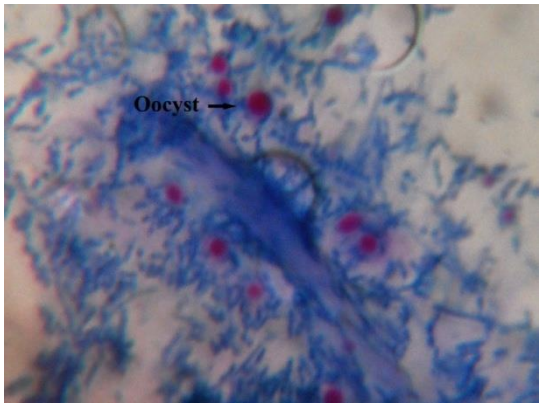

Fig. 1: Oocysts of Cryptosporidium parvum in modified Ziehl-Neelsen staining (X1000)

Discussion

Intestinal protozoan parasitic infections are the commonest and major cause of morbidity and mortality in HIV positive cases World wide[7]. These organisms usually cause a self limiting illness in immunocompetent individuals but in the case of immunocompromised patients they can cause life threatening, profuse watery diarrhea[1,8]. The present study documents that infections with intestinal protozoan parasites are common in HIV seropositive individuals. Ayyagari et al has reported a high prevalence of Isospora belli from the Northern parts of India[9], where as Kumar et al has reported Cryptosporidium parvum as the commonest parasite which was associated with HIV infections in Southern parts of India[10]. Our study projects Cryptosporidium parvum as the most common coccidian parasite which was associated with immunocompromised cases, both with diarrhoea and without diarrhoea. In our study also high prevalence of Cryptosporidium parvum (47.50%) was observed. While Gupta et al[7] and Mukhopadhya et al[11] have reported a low prevalence of Cryptosporidium parvum as compared to Isospora belli. The Ziehl-Neelsen staining technique had fewer steps than some of the other staining methods and could be used in a parasitological laboratory. It was less expensive, samples could be used in batches and the stained slides could be kept as a permanent record[6]. Modified Ziehl-Neelsen staining shows oocysts of Cryptosporidium, X1000 [Fig. No: 1].

The newer methods become available for the laboratory diagnosis of cryptosporidiosis including the currently available second generation immunofluorescence, ELISA and molecular methods help in the detection of oocysts. A high prevalence of intestinal parasitic infections in HIV positive individuals may be due to contaminated water supply and lack of personal hygiene, which is common in a rural scenario.

However the techniques which were employed for diagnosing the common parasitic infections are simple and rapid as compared to those which are used for bacterial, fungal and viral infections. But timely diagnosis is hampered by the delay in the diagnosis of HIV and the lack of resources for its investigation in rural parts developing countries. Patients from the rural areas belong to a poor socio-economic background and cannot afford diagnosis and treatment. The authors suggest that along with early diagnosis and treatment and other steps should be taken to improve the water supply, sanitation and to provide health education to prevent the morbidity and mortality which are associated with HIV infected individuals and also immunocompromised cases.

Conclusion

Cryptosporidium parvum is major water borne protozoan pathogen; water contamination should be investigated to protect public health from the risk of transmission of the pathogen. A modified Ziehl-Neelsen staining is selected to stain faecal smears.

Acknowledgement

We thank all clinicians of our Hospital for early diagnosing and treating intestinal parasitic infection cases.

References

1. Deorukhkar S,Katiyar R, Saini S, Siddiqui AV, The prevalence of intestinal parasitic infections in HIV infected patients in a rural tertiary care Hospital of Western Maharashtra, Journal of Clinical and Diagnostic Research,2011; 5(2): 210-212.
2. Becker ML, CohenCR, MaryCheang, Reynold GW, Blanchard JF, StephenMoses, Diarrheal disease among HIV infected adults in Karnataka, India: Evaluation of risk factors and aetiology, The American Journal of Tropical Medicine and Hygiene, 2007;76(4):718-722.
3. SitaraSwarna RA, PremiSankaran, Gagandeep Kang, Cryptosporidium species in HIV infected individuals in India: An overview, The National Medical Journal of India, 2008; 21(4):178-184.
4. Ortolani EL, Standardization of the modified Ziehl-Neelsen technique to stain oocysts of Cryptosporidium species, Rev Brazil Journal Vet Parasitol, 2000; 9(1): 29-31.
5. Karyakarte R, Damle A, Medical Parasitology, In: Other members of Phylum Apicomplexa, Chapter 6, Arunbhasen Book and Allied(P) Ltd, Kolkata; 2005:91.
6. MacPherson DW, McQueen R, Cryptosporidiosis: Multiattribute evaluation of six diagnostic methods, Journal of Clinical Microbiology, 1993; 31(2): 198-202.
7. Gupta S, Narang S, Nunavath V, Singh S, Chronic diarrhoea in HIV patients: Prevalence of coccidian parasites, Indian J Med Microbiol, 2008; 26(2): 172-175.
8. Meisel JF, Perera DR, Meligrilo C, Rubin CB, Overwhelming watery diarrhoea associated with Cryptosporidium an

immunosuppressed patients, Gastroenterology, 1976; 70: 1156-1166.
9. Ayyagari A, Sharma AK, Prasad KN, Dhole TN, Kishore J, Chaudhary G, Spectrum of opportunistic infections in Human Immunodeficiency Virus infected cases in a tertiary care Hospital, Indian J Med Microbiol,1999; 17(2): 78-80.
10. Kumar SS, Ananthan S, Lakshmi P, Intestinal parasitic infections in HIV infected patients with diarrhoea in Chennai, Indian J Med Microbiol, 2002; 20(2): 88-89.
11. Mukhopadhya A, Ramakrishna BS, Kang G, Pulimood AB, Mathan MM, Zachariah A, Mathai DC, Enteric pathogens in Southern Indian HIV infected patients with and without diarrhoea, Indian J Med Res, 1999; 109(March): 85-89.

Conflict of Interest: Nil
Source of support: Nil