



International Journal of Preclinical & Pharmaceutical Research

Journal homepage: www.preclinicaljournal.com

DETECTION OF *CRYPTOSPORIDIUM PARVUM* OOCYSTS BY MODIFIED ZIEHL-NEELSEN STAINING IN HIV/AIDS INDIVIDUALS AT A TERTIARY CARE HOSPITAL

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ABSTRACT

Diarrhoea is a common complication of infection with HIV/AIDS leading to weight loss. It occurs in almost 90% of the 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. To detect oocysts of *Cryptosporidium parvum* by modified Ziehl-Neelsen staining in HIV/AIDS patients attending our Hospital. Stool samples from Fifty one HIV patients with diarrhoea and one HIV patient without diarrhoea were examined for oocysts by modified Ziehl-Neelsen (ZN) staining. Of the 52 stool samples, modified ZN staining detected 14 (26.92%) stool samples positive for oocysts of *Cryptosporidium parvum*. Conclusions: There is a high prevalence of cryptosporidiosis in HIV /AIDS 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 are 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. Parasitic infections with *Isoospora 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 intra cellular 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. Identification of oocysts in stool samples has been described, modified Ziehl-Neelsen (ZN) staining (1% sulphuric acid must be used) [4,5].

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

MATERIAL AND METHODS

A retrospective study was carried out in the Department of Microbiology for a period of one year (October 2010 to September 2011). Fifty two stool samples

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from HIV positive 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 10ml 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 52 HIV seropositive cases were studied, 51 cases (98.08%) presented with diarrhoea where as one case (1.92%) did not have diarrhoea. The number of HIV infected males presenting with diarrhoea was more than that of the females [Table No: 1]. The prevalence of *Cryptosporidium parvum* in our study is 32.69% (17/52) [Table No: 2]. The number of stool samples considered positive in our study was 17 out of 52 samples.

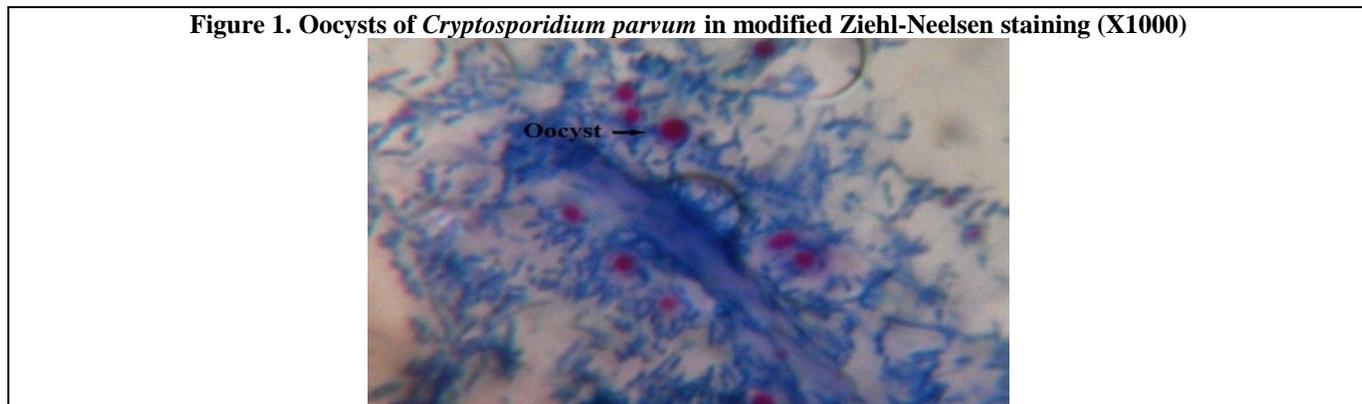
Table 1. Gender and Diarrhoea wise distribution of HIV positive cases (n=52)

Gender	With diarrhoea	Without diarrhoea	Total
Male	30	01	31
Female	21	00	21

Table 2. *Cryptosporidium parvum* Oocysts positive in HIV cases

No. Stool tested	No. Positive	Percentage
52	17	32.69

Figure 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 Worldwide [6]. 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 [1,7]. 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 [8], where as Kumar et al has reported *Cryptosporidium parvum* as the commonest parasite which was associated with HIV infections in Southern parts of India [9].

Our study projects *Cryptosporidium parvum* as the most common coccidian parasite which was associated with HIV positive cases, both with diarrhoea and without diarrhoea. In our study also high prevalence of *Cryptosporidium parvum* (32.69%) was observed. While

Gupta et al [7] and Mukhopadhyaya et al [10] 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 [11]. Modified Ziehl-Neelsen staining shows oocysts of *Cryptosporidium*, X1000 [Figure 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 author suggests 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.

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.

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