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Original Research Article

Coccidian parasitic infection in HIV positive patients attending Tertiary care Hospital in Nepal

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Abstract

Background: Enteric opportunistic parasites are the leading cause of gastrointestinal tract infection in patients with HIV/AIDS. This study was carried out to establish the occurrence of intestinal coccidian parasitic infection among the HIV positive patients in Eastern Nepal.

Materials and method: This was a hospital based study carried out in Microbiology laboratory, BPKIHS, Dharan, Nepal over a period of one year (May 2013 to April 2014). All HIV positive patients with CD4+ count ≤200cells/µl were included. Total 50 HIV positive patients participated in the study over one year period. Stool sample was collected after taking an informed written consent from the patient. Isolation and identification was done as per standard Microbiological procedure.

Result: The overall prevalence of intestinal parasitic infection in present study was 38%. Among the parasites, coccidian parasites comprised of 57.89% of total parasitosis, among 20% (n= 10) cases. Out of total coccidian parasites identified *Cryptosporidium parvum* comprised 72.7%, *Cyclospora cayetanensis* 18.2% and *Isospora belli* 9.1%. One patient had mixed Cryptosporidial and *Cyclospora* infection.

Conclusion: Coccidian parasitic infections are common in HIV positive patients at lower CD4+ count in Eastern part of Nepal.

Keywords: HIV, opportunistic infections, coccidian parasite.

1. Introduction

HIV infection leading to AIDS continues to be a major health problem with 2.3 million new cases and 1.6 million AIDS related death in 2012 globally [1]. Since 1988 when the first case of HIV infection was detected in Nepal, there has been steady rise in HIV infected population [2].

Since the beginning of the AIDS pandemic, Opportunistic infections (OIs) have been recognized as widespread complications of HIV infection [3]. Gastrointestinal infections are very common in patients with HIV/AIDS [4]. Diarrhoea is the most frequent clinical presentation of these infections. Diarrhoea occurs in 30-60% of AIDS patients in developed countries and in about 90% of AIDS patients in developing countries [5].

The opportunistic intestinal coccidian parasites *Cryptosporidium parvum, Cyclospora cayetanensis, Isopora belli* are documented in patients with HIV/AIDS [2, 6]. Other enteric parasites like *Entamoeba histolytica, Giardia lamblia, Strongyloides stercoralis, Trichuris trichuria, Anchylostoma duodenale, and Ascaris lumbricoides* are frequently encountered in AIDS patients in developing countries [5].

CD4+ T-lymphocytes play a central role in regulation of immune response. The progressive depletion of CD4+ T lymphocytes in HIV infection increases the risk and severity of opportunistic infections which are the leading cause of morbidity and mortality in these patients [6].

Majority of opportunistic infections occurs when CD4+ T-cell count is below 200cells/µl [5, 7, 8]. So, with this study we have made an attempt to find out the prevalence of intestinal coccidian parasitic infection amongst the HIV positive patients with CD4+ count below 200cells/µl in Eastern Nepal. This study will help in establishing the magnitude of problem and also in the management, both in the treatment as well as in the prophylaxis against these infections.

2. Method

This study was carried out in the department of Microbiology, BPKIHS, Dharan, Nepal from May 2013 to April 2014). Ethical clearance was taken from the institutional review board and informed written consent from the patients.

Total 50 HIV positive patients whose CD4+ count was ≤200cells/µl and who gave the consent participated in the study. Before sample collection participants name, age, sex, history of diarrhea, drug treatment history like antiretroviral therapy, co-trimoxazole (Trimethoprim-sulphamethoxazole), antiparasitic, antibiotic was taken through questionnaire. Three stool containers with participant's identification number and with instructions for stool collection were given irrespective of sign and symptoms of gastrointestinal tract infection. Stool sample from an equal number of age-matched HIV negative patients was taken as control.

Macroscopically consistency of stool was noted; presence of blood or mucus was noted. For microscopy first the wet mount of the faeces was done i.e. saline wet mount and iodine wet mount. Then formalin ethyl-acetate concentration technique was done. To identify the oocyst of *Cryptosporidium, Cyclospora and Isospora belli*, Modified Ziehl-Neelsen staining method was performed: All the stained smears were screened for oocysts of *Cryptosporidium parvum, Isospora belli and Cyclospora cayetanensis. Cryptosporidium* oocysts were identified as modified acid fast stain positive oval to round with size 4-6 µm with or without the presence of retracted cytoplasm. *Cyclospora* were identified as acid fast round irregularly stained structure 8-10 µm in size with crumpled celophane appearance. Similarly *Isospora* oocysts were identified as modified acid fast stain positive oval structure 20-30 µm by 10-19 µm in size. [9]. Stool samples from HIV negative cases were also screened.

CD4+ count of the patients was done by FACS (Becton and Dickinson).

3. Result

Stool samples were submitted by 50 (150 stool samples) patients for investigation. Among them majority of the patients were male (32/50). The mean age of the participants was 34.03 year (SD±5.0). The most common mode of HIV transmission in the studied population was sexual contact (38/50) followed by intravenous drug abuse (4/50). Out of 50 patients who submitted their stool sample 26 patients had symptomatic diarrhea.

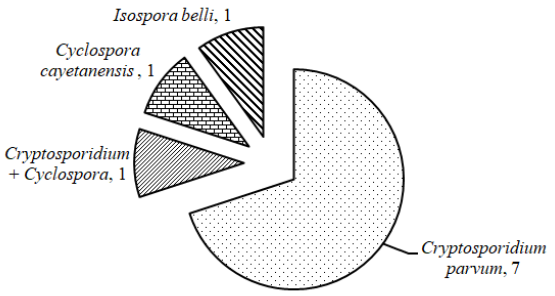
Overall 38 % (19/50) of patients were excreting the ova/cyst of parasites in their stool identified in stool microscopy (table 1).

Table 1: Ova/cyst of intestinal parasites observed in microscopic examination of stool samples of HIV positive patients

Intestinal parasites isolated	Number
Cryptosporidium parvum	8
Giardia lamblia	4
Cyclospora cayetanensis	2
Entamoeba histolytica	2
Isospora belli	1
Stongyloides stercoralis	1
Hookworm	1
Total	19

Intestinal coccidian parasitic infection was seen in 20% (10/50) of patient which comprised 57.9% (11/19) of total parasites identified. Among the coccidian parasites positive patients *Cryptosporidium* infection was most frequent as shown in figure 1.

Figure 1: Distribution of cases with coccidian parasites in stool sample



Cryptosporidium parvum comprised 72.7%(n=8) of the coccidian parasites identified amongst the patients having CD4+ range 50-150 cells/µl while *Cyclospora cayetanensis* comprised 18.2% (n=2) amongst patients with CD4+ count in the range of 151-200 cells/µl and Isospora belli 9.1%(n=1) in patient with CD4+ count 10cells/µl as in shown in table 2. Four patients with *Cryptosporidium parvum* infection was also co-infected with cyclospora (1), Entamoeba histolytica (2), Giardia lamblia (1).

Table 2: Distribution coccidian parasites with their CD4+ range

Type of infection	Frequency	Percentage (%)	CD4+ range Cells/µl
Cryptosporidium parvum	8	72.7	50-150
Cyclospora cayetanensis	2	18.2	151-200
Isospora belli	1	9.1	10
Total	11	100	

Nine out of 10 patients suffering from intestinal coccidian parasitic infection had symptomatic diarrhea and one patient was asymptomatic which was statistically significant ($p<0.001$) as shown in table 3

Table 3: Showing association of diarrhea and the coccidian parasites

		Coccidian parasites				
		Cryptosporidium parvum	***Cyclospora cayetanensis***	***Isospora belli***	***Cryptosporidium + cyclospora***	**Total**
Diarrhea	Ab	1	0	0	0	1
	P	6	1	1	1	9
Total		7	1	1	1	10

Ab: Absent; P: Present

No significant variation was found among the coccidian parasite positive patients who were ART naïve and those on ART ($p=0.426$). Association between the sources of drinking water and coccidian parasites identified could not be established.

None of the stool samples from HIV negative patients taken as control was positive for coccidian parasite.

4. Discussion

Gastrointestinal parasitic infection is one of the major problems and remains an important cause of morbidity and mortality among the HIV infected individuals in developed as well as in developing countries [2, 10]. The coccidian parasites *Cryptosporidium* species, *Cyclospora* species, *Isospora belli* are the foremost among the enteric parasites in these patients. In case of immunocompetant patients it causes self-limiting infection but as the immunity decreases, they cause life-threatening infection [11]. The overall prevalence of parasitic infection in present study was 38% (19/50). This supports the finding of study in Pune, India where it was 35% [5]. Likewise lower prevalence have also been reported in Western Nepal (22.4%) [12] and higher prevalence in Central Nepal (67.4%)[10] study compared to the present study.

Earlier report from Eastern Nepal [2] have reported intestinal coccidian to be 52.8% of total intestinal parasites identified this matched with our present study where it was found to be 57.9%. In study conducted in New Delhi, India [11] it was identified in 76.3% while two studies conducted in North India [2] and Central Nepal [10] have reported 36% and 30% respectively lower than the present study.

Present study reports 72.7% of *Cryptosporidium* among the coccidian parasites isolated. Earlier study done in Eastern and Central Nepal [2, 10] has noted isolation of *Cryptosporidium* 50% and 30.7% while study in Pune India [5] isolation was 59%. Cryptosporidiosis, tend to vary from one locality to another and from one country to another depending on the level of contamination in the water, food and contact with animals, which are important factors in the dissemination of the parasite. Also, the small size (3-5 µm) of oocysts, their resistance to chlorine disinfections, and low infective dose are the major infective potential of *Cryptosporidium parvum* [7].

Cyclospora cayetanensis was second most coccidian (18.2%) in our study while study in Central Nepal [10] documented it to be most common coccidian than *Cryptosporidium parvum*. Although *Cyclospora* is endemic in Nepal [2] the prevalence of *Cyclospora* (18.2%) in the present study was lower than the previous study in Eastern [2] and Western Nepal [13] which showed 40% and 37.8%.

Higher preponderance of *Isospora belli* than the other coccidian parasites has been observed in study in North (50%) [11] and South India (18.6%) [14]. However it was observed only in one patients comprising 9.1% of coccidian parasite identified in this study. The frequency of Isosporiasis in AIDS is likely to be underestimated due to asymptomatic shedding of oocyst and treatment with trimethoprim-sulphamethaxazole for other infections in AIDS cases which may confer some protection against this protozoan [14]. Opportunistic coccidian parasitic infection can be acquired any time during the course of HIV infection but the establishment of infection occurs when the CD4+ count is less than 200 cells/µl [11] also shown by reports [5,7]. In our study the CD4+ range of the patients with Cryptosporidial infection was 50-150 cells/µl, *Cyclospora* was seen in the CD4+ range of 151-200 cells/µl while CD4+ count of patients with *Isospora* infection was 10cell/µl.

Nine out of 10 patients suffering from intestinal coccidian parasitic infection had symptomatic diarrhea which was statistically significant ($p<0.001$) similar significant association was found in a study in India [3, 14]. Thus infections with opportunistic pathogen are leading cause of diarrhea in patients with advanced stage of disease [5].

The difference in the prevalence of the parasitic infection may occur due to difference in the endemicity of parasites, sanitary practices and level of education, economic status and social behavior [10].

Mixed infections are common among the HIV/AIDS patients [2, 11]. In this study mixed infection with *Cryptosporidium*, *Entamoeba histolytica* was seen in two patients, *Cryptosporidium*, *Giardia lamblia* was seen in one patient and *Cryptosporidium* and *Cyclospora* was seen in one patient while no mixed infection was seen with *Isospora belli*.

Antiretroviral therapy is the best way to improve the immune status in HIV infected patients and thus avoids the potentially fatal opportunistic infections [2]. Among the coccidian parasite positive patient seven patients were ART

naïve while three patients were on ART and no significant variation was found coccidian parasitic infection among the patients who were ART naïve and those on ART. This might be due to non-compliance of the drug and difficulty in accessing the anti-retroviral distribution site as most of the patients in our study were referred from primary health centre.

5. Conclusion

Opportunistic coccidian parasites like *Cryptosporidium parvum, Cyclospora* are common in HIV positive patients and are the leading cause of diarrhea in these patients in Eastern part of Nepal and The constant monitoring of these infections in HIV positive patients is important for prevention and better management.

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