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Research Article

SPECTRUM OF INTESTINAL COCCIDIAN PARASITES AMONG HIV PATIENTS ATTENDING A TERTIARY CARE HOSPITAL IN NORTH INDIA

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ABSTRACT

Objectives: Human immunodeficiency virus (HIV) infection severely compromises the immune system, leading to increased susceptibility to opportunistic infections. Intestinal parasitic infections, particularly those caused by coccidian parasites, significantly contribute to morbidity and mortality in HIV-infected individuals, with diarrhea being one of the most common clinical manifestations.

Methods: The study involved HIV seropositive and seronegative patients presenting with diarrhea and other gastrointestinal symptoms. Stool samples were analyzed using direct wet mount preparations and modified acid-fast staining to identify various intestinal coccidian parasites among HIV patients and their prevalence.

Results: Among 207 HIV seropositive patients tested, 62% were male, with ages ranging from 11 to 60 years. *Cryptosporidium* was the only coccidian parasite detected in our study, with a higher prevalence (77%) in patients with chronic diarrhea compared to those with acute diarrhea (23%). A significant majority of parasites were found in patients with cluster of differentiation (CD) CD4 counts below 200/mm^3.

Conclusion: There is a high prevalence of intestinal coccidian parasites among the studied population. Health education targeted at both HIV patients and community health workers regarding potential sources and transmission routes of these infections is crucial to reduce contamination and promote patient health and longevity.

Keywords: Human immunodeficiency virus-acquired immunodeficiency syndrome, Intestinal coccidian parasites, *Cryptosporidium*, Diarrhea, Opportunistic infections.

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INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) is a major global public health problem caused by the human immunodeficiency virus (HIV). An estimated 39 million people worldwide are living with HIV infection [1]. The unique pathogenesis of HIV mainly affects the CD4+ T helper cells, making opportunistic infections a common and severe issue, significantly impacting the morbidity and mortality of infected individuals. Diarrhea is often a clinical presentation of such infections and can become life-threatening [2-4].

As per the World Health Organization (WHO), diarrhea is defined as the passage of three or more loose or liquid stools per day (or more frequent passage than is normal for an individual) [5].

Opportunistic parasitic infections, including protozoa and helminths, are neglected tropical diseases that are targeted by sustainable development goals to be eliminated by 2030 [3]. The coccidian parasites Cryptosporidium species, Isosporabelli, Cyclospora species, and *Microsporidia* species are the most common enteric parasites in immunocompromised patients that can usually lead to lethal severe diarrhea, which are otherwise considered causative agents of mild and self-limiting gastrointestinal disorders in immunocompetent individuals [6].

Acute or chronic diarrheic syndromes resulting from these coccidian parasites are usually accompanied by weight loss, dehydration, abdominal pain, and malabsorption syndrome in immunocompromised patients. Chronic diarrhea can lead to an increase in morbidity and mortality in these patients. Diarrhea with fluid loss of 25 L/day has been seen in infected patients, which can persist for weeks in immunocompromised patients [6].

The high mortality rate due to intestinal coccidian parasitic infection among HIV patients warrants attention toward a probable link between provisions of unsafe drinking water, poor sanitation, and the incidence of diarrheal diseases, particularly in rural areas [7].

Therefore, the present study aims to identify various intestinal coccidian parasites among HIV patients presenting to the department, so as to facilitate awareness and health education of the local population and for clinicians about the importance of testing and diagnosing coccidian parasitic infections on a routine basis.

METHODS

A retrospective, cross-sectional, observational analysis was conducted in the Department of Microbiology, Government Medical College, Amritsar spanning from January 2016 to June 2023 after obtaining approval from the Institutional Ethical Committee.

Inclusion criteria

The previously tested HIV seropositive patients referred from the antiretroviral therapy center with complaints of persistent or chronic diarrhea and HIV seronegative patients referred from the general outpatient department, who were simultaneously tested for viral markers in the microbiology department and tested negative, also with complaints of diarrhea and other gastrointestinal symptoms, with ages ranging from 11 to 60 years, were included.

Exclusion criteria

Patients who had taken antibiotics, antiprotozoal, or antimotility drugs in the preceding 2 weeks were excluded.

All the tests were done after due patient consent. HIV seropositive patients were defined as those who had tested positive for HIV antibodies by two sequential enzyme-linked immunosorbent assay/rapid tests as per the recommendations given by the WHO.

Stool samples were received in a single, clean, dry, wide-mouthed container, both from HIV-seropositive as well as HIV seronegative patients. Diarrheal stool samples of patients were examined by direct wet mount preparations using normal saline to detect motile trophozoites and lugol's iodine to detect ova, larva, and cysts. Air-dried smears from fresh stool samples were fixed and stained by a modified acid-fast stain using 1% acid-alcohol as a decolorizer to detect the coccidian parasites; *Cryptosporidium, Isospora, and Cyclospora* species. Furthermore, slides were extensively studied for variations in size, shape, internal structure, and staining characteristics. Micrometry was performed to ascertain the exact oocyst size.

RESULTS

The study encompassed 207 HIV-positive patients, with a male predominance (62%). The age range was 11–60 years. Stool samples from 225 HIV seronegative patients, were also tested during this period.

The prevalence of intestinal coccidian parasites among HIV seropositive and HIV seronegative patients is depicted in Table 1 and Table 2, respectively.

Among HIV seropositive patients, *Cryptosporidium* species 100% (132/132) was the only coccidian parasites identified in our study, predominantly in patients with chronic diarrhea and those with severely compromised immune systems (CD4<200/mm^3).

The oocysts load was 3 to >150 per high power field. The average size of *Cryptosporidium* species oocysts ranged from 3.95 to 6.09 μm with a mean of 5.43 μm [8]. The majority of the oocysts were uniformly stained; a few were non-uniformly stained. The shape of these oocysts was generally round [8].

The most common species of *Cryptosporidium* infecting humans are *Cryptosporidium hominis* and *Cryptosporidium parvum* with the size of oocysts measuring 4.9×5.2 μm and 5.0×4.5 μm [9], respectively. The internal structures could not be made out with modified acid-fast staining to comment upon various species of *Cryptosporidium* seen in this study.

The prevalence of diarrhea among HIV seropositive patients, who test positive for coccidian parasites is depicted in Table 3. Intestinal coccidian parasites were detected more commonly in patients with chronic diarrhea (77%) than in patients with acute diarrhea (23%). The maximum (90%) number of coccidian parasites were detected among patients with CD4 counts < 200 cells/mm^3 as depicted in Table 4.

DISCUSSION

The high incidence of *Cryptosporidium* among HIV-infected patients highlights the vulnerability of this group to intestinal coccidian parasites, exacerbated by their immunocompromised status. Environmental factors, such as poor sanitation and hygiene, also contribute significantly to the transmission and prevalence of these parasites [10].

In the present study, the overall prevalence rate of coccidian infection among HIV seropositive individuals was 63.7% (132/207). This result is higher than the earlier reports in different parts of India: 29% from New Delhi [11], and 18% in Rajasthan [12]. The prevalence difference might be due to the geographic difference, sensitivity of diagnostic techniques, study participants' immunity status, hygiene practice, socioeconomic difference, change in living conditions, and awareness of patients on coccidian parasitic infection of the study participants [10].

Table 1: Prevalence of coccidian parasites among HIV seropositive patients

HIV seropositive	Coccidian parasite	
Number (n)	**Number (n)**	**Percentage**
207	132	63.7

Table 2: Prevalence of coccidian parasites among HIV seronegative patients

HIV seronegative	Coccidian parasite	
Number (n)	**Number (n)**	**Percentage**
225	0	0

Table 3: Prevalence of diarrhea among HIV seropositive patients who test positive for coccidian parasites

Symptom	Number (n)	Percentage
Acute diarrhea	30/132	23
Chronic diarrhea	102/132	77

Table 4: Coccidian parasite and CD4 cell count among HIV seropositive patients

CD4 cell count	Positive for Cryptosporidium oocyst Number (n=132)	Percentage
CD4<200	119	90
CD4=200	13	10
CD4>200	0	0

Among HIV seropositive patients, males (62%) were more affected than females (38%). The age group of the patients affected was from 11 to 60 years. This study is in concordance with the study done by Namaji *et al*. [13] and Chandi *et al*. [14].

In our study, *Cryptosporidium* species (100%) was the only parasite detected in HIV-positive patients with diarrhea. This study corresponds with the study done by Getachew *et al*. [10] and Namaji *et al*. [13]. Biological factors that impact the epidemiology of *Cryptosporidium* include their low infective dose (10–100 oocysts), ubiquitous nature, small (4–5 μm) size, environmentally and chemically resistant sporulated/infectious oocysts when passed in stool [13].

Other similar studies conducted at various reference and tertiary care centers in New Delhi [11], Rajasthan [12], Bihar [15], and Mumbai [16] showed infection with Isospora belli, Cyclospora, Microsporidia and also increased rate of mixed parasitic infection with *Isospora belli and Cryptosporidium* species was observed, which was not detected in our study. This difference in the findings may be attributed to geographical variation, as well as differences in the populations, socioeconomic status and access to potable water, their personal hygiene, and sanitary habits, different screening, staining methods, and molecular methods for detection of parasites used by several studies leading to variation in percentage and pattern of parasites identified [10].

CONCLUSION

Intestinal coccidian parasites pose a significant health risk to HIV-infected patients. The study underscores the importance of continuous monitoring and health education regarding the prevention and

management of parasitic infections, which is vital for improving patient outcomes and reducing the transmission of these infections.

CONFLICTS OF INTEREST

There are no conflicts of interest.

AUTHORS CONTRIBUTION

All the authors contributed equally to concepts, design, data analysis, manuscript preparation, manuscript editing, and review of the article.

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