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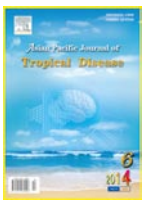

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Spectrum of opportunistic and other parasites among HIV/AIDS patients attending a tertiary care hospital

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PEER REVIEW

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Comments

This is a good study in which the authors have noted the spectrum of opportunistic and other parasites among HIV positive patients. These infections were also correlated with the absolute CD4 count ranges in which they occurred maximally. Thus proving the data regarding the prevalent pathogens and the CD4 counts where they are expected to be present maximally, a keen lookout for these pathogens can be made. This will help to alleviate the morbidity of the HIV infected patients.
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ABSTRACT

Objective: To determine the spectrum of opportunistic as well as non-opportunistic parasitic infections in HIV/AIDS patients.
Methods: A total of 250 HIV sero-positive individuals are included in study. Among them, 76 clinical cases of diarrhea and 8 clinically suspected cases of toxoplasmosis were identified. Fresh stool samples were collected in a suitable container on three consecutive days and processed immediately for identification of oocysts of *Cryptosporidium parvum*, *Isospora belli* and *Cyclospora*. Blood sample was collected from suspected cases of toxoplasmosis and tested for antitoxoplasma immunoglobulin M antibodies using immunoComb Toxo IgM test. Estimation of CD4 counts was also done by flow cytometry from these patients.
Results: The opportunistic parasites identified in total HIV sero-positive patients were *Cryptosporidium* spp. (20.8%) and *Isospora belli* (0.8%). While the non-opportunistic parasite identified were *Entamoeba histolytica* (4%), *Giardia intestinalis* (1.6%) and *Hymenolepis nana* (0.8%). Toxoplasmosis was identified in 2.4% HIV sero-positive patients.
Conclusions: Increasing prevalence of parasitic infections in HIV/AIDS patients suggests that simple steps such as drinking safe water, maintaining high level o=f environmental and personal hygiene and avoiding contact with contaminated soil are necessary to prevent the occurrence of these diseases in AIDS patients

KEYWORDS

Opportunistic parasitic infections, *Cryptosporidium* spp., *Isospora belli*, *Entamoeba histolytica*, HIV/AIDS

1. Introduction

The burden of HIV/AIDS has increased enormously worldwide, especially in developing countries. The estimated burden in India alone was 2.1 million in 2011, which was about 7.2% of global burden[1]. Children whose age is younger than 15 years old account for 7% while 86% were adults in the age groups of 15-49 years old with 39% women of all HIV infections[1]. The morbidity and mortality associated with HIV infections is very high and it is not because of virus alone but also due to opportunistic infections associated with the disease[2]. Since the immune system is severely affected in HIV infections, organism with low-virulence becomes pathogenic to cause severe life threatening diseases. Opportunistic parasites that cause self-limited illness in immunocompetent individuals can cause intractable, prolonged and severe diarrhea leading to weight loss and cachexia in HIV infected patients. Opportunistic parasitic infections are very common in HIV/AIDS patients with CD4 count <200 cell/mm^3 as it creates a

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suitable environment for intestinal parasites[3,4].

Opportunistic intestinal parasites frequently isolated from HIV infected patients include *Cryptosporidium* spp., *Cyclospora cayetanensis*, *Isospora belli* (*I. belli*), *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*[5,6]. Parasites are commonly reported in patients from developing countries but currently not considered as opportunistic include *Entamoeba histolytica* (*E. histolytica*), *Giardia lamblia*, *Ascaris lumbricoides*, *Ancylostoma duodenale*, *Strongyloides stercoralis* and *Trichuris trichiura*[5,6]. Intestinal parasites are widely distributed in developing countries such as India mainly due to poor sanitation, low-level environmental hygiene, improper waste and human excreta disposal resulting in contamination of drinking water and food[7].

Toxoplasmosis in HIV/AIDS patients is primarily due to reactivation of latent infection as a result of decreased level of immunity[8]. Clinical manifestations are more serious in immunocompromised patients in comparison to immunocompetent individuals. The most common brain lesion seen in AIDS is toxoplasmic encephalitis[9,10]. A clinically suspected case needs confirmation by radiological and immunological findings such as specific antibody detection. Anti-toxoplasma immunoglobulin M (IgM) antibody titres usually rise during active phase of infection and fall within few months[11].

Clinical presentation of AIDS and the prevalence of various opportunistic parasites may vary in frequency in different countries and even in different regions within the country. The knowledge of prevalence of such opportunistic parasites is necessary for proper management of HIV/AIDS case. Thus the present study was aimed to determine the spectrum of both opportunistic and non-opportunistic parasites infecting HIV/AIDS patients in a major tertiary care setup.

2. Materials and methods

The present study is conducted in Department of Microbiology, Government Medical College, Aurangabad, India during the period of January, 2007 to October, 2008. A total of 250 HIV sero-positive patients either admitted in the hospital or attended the Antiretroviral Therapy Centre were included in the study. HIV status of the patients was confirmed by guidelines provided by NACO (Strategy III). Among them clinical cases of diarrhea were examined for demonstration of intestinal parasites. Fresh stool samples were collected in a suitable container on three consecutive days and processed immediately after collection. Saline and iodine wet mount and 3 smears were prepared from each sample. The smears were stained with modified cold Kinyouns method for demonstration of oocysts of *Cryptosporidium parvum*, *I. belli*, and *Cyclospora*.

Five milliliters of blood was collected from clinically and radiologically suspected cases of toxoplasmosis under universal aseptic precautions by a venepuncture in a sterile plain glass vial and serum was separated. The serum sample was then tested for anti-toxoplasma IgM antibodies using immunoComb Toxo IgM test as per the protocol (Orgenics Ltd.). Three milliliters of blood was also collected in an EDTA vacutainer for estimation of CD4 counts by flow cytometry from these patients.

A proper ethical clearance has been taken from the ethical committee to conduct the study. All experiments were carried out in compliance with the relevant laws and guidelines in accordance with the ethical standards of the Declaration of Helsinki.

3. Results

Among 250 HIV sero-positive patients, 76 clinical cases of diarrhea and 8 clinically suspected cases of toxoplasmosis were identified. Of 76 cases of diarrhea, intestinal parasites were detected in 70 (92.1%) patients. The opportunistic parasites identified in patients of diarrhea were *Cryptosporidium* spp. and *I. belli*. While the non-opportunistic parasite identified were *E. histolytica*, *Giardia intestinalis (G. intestinalis)* and *Hymenolepis nana* (*H. nana*). Table 1 shows the frequency of occurrence of intestinal parasites in such patients. *Cryptosporidium* spp. was found to be the most common parasites among intestinal pathogens. Among the 8 suspected cases of toxoplasmosis 6 patients (prevalence 2.4%) shows a rise in titre of anti-toxoplasma IgM titres.

Table 1

Distribution of different intestinal parasites among HIV positive patients.

Isolated pathogens		No. of parasites	Positivity*	Prevalence#
Opportunistic parasites	*Cryptosporidium* spp.	52	74.28%	20.8%
	I. belli	2	2.85%	0.8%
Non-opportunistic parasites	*E. histolytica*	10	14.28%	4.0%
	G. intestinalis	4	5.71%	1.6%
	H. nana	2	2.85%	0.8%
Total		70	100.00%	28.0%

*: Percentage among the total number of parasites.
#: Percentage among the total number of HIV patients.

CD4 counts to determine the most common range in which various parasitic infections occurred are (360.0±85.1) and (86.0 ±11.4) in chronic diarrhea and toxoplasmosis, respectively (grading of CD4 counts range done according to the Centers for Disease Control and Prevention guidelines)[12]. Statistical analysis was performed using *Chi* square tests and a *P* value calculated. A *P* value of <0.05 was obtained, thus validating the results.

4. Discussion

The majority of morbidity and mortality in HIV infected patients with clinical stage 3 and 4 is accounted for by the opportunistic infections which opportune upon the lowered cellular and humoral defense artillery of the infected individuals. A wide variety of these infections presenting a myriad of organisms including bacteria, fungi, viruses and parasites are encountered in the AIDS population[13]. Gastrointestinal infections are the common infections seen in immunocompromised patients caused mainly by opportunistic intestinal parasites.

Intestinal parasitic infections were observed in 28% (70/250) of the patients in our study. A similar high prevalence of intestinal parasitic infections were noted by Kulkarni *et al.* (35%), Ramana *et al.* (44.6%), Pavie *et al.* (17%), Zeynudin

et al. (39.56%)[3,5,14,15]. Among the opportunistic intestinal parasites isolated, the majority of infections was contributed to *Cryptosporidium* sp. (74.28% of stool samples, 20.8% prevalence) followed by *Isospora* (0.8%). Such a dominance of cryptosporidial diarrhea was reported by several other studies[5,16,17]. While non-opportunistic parasites identified were *E. histolytica* (4%), *G. intestinalis* (1.6%) and *H. nana* (0.8%). Poor personal hygiene, low socioeconomic status and contaminated drinking water may be responsible for the high frequency of *Cryptosporidium* in our and other studies conducted in India. An increasing prevalence of *I. belli* infection among HIV/AIDS patients with diarrhea was observed by Pavie *et al*, Kulkarni *et al.* and Alemu *et al*[3,15,18]. However, a very low prevalence was seen in this study.

In this study about clinical findings, radiological features and IgM anti-toxoplasma antibody were used to establish the diagnosis of toxoplasmosis. The prevalence of cerebral toxoplasmosis as determined by IgM anti-toxoplasma antibody test in our study was 2.4%. Malla *et al* and Walle *et al* also applyed IgM detection for diagnosis of toxoplasmosis in HIV sero-positive patients and reported a prevalence of 6% and 10.7% respectively[11,19]. Sharma *et al* have reported a prevalence of 3.7% of toxoplasmosis[20]. A lower prevalence was reported by Chakravarty *et al* (0.7%)[21].

The pre-existing toxoplasmosis is denoted by a specific immunoglobulin G antibody response while IgM usually denotes active infection or reactivation of latent infection in HIV infected patients. However, due to suppressed immune status the antibody responses in HIV/AIDS patients are often low or even may not be detectable, making it difficult to diagnose and treat the disease. However, in a study conducted in France, where toxoplasma infection is highly prevalent, patients with acute toxoplasmic encephalitis demonstrated specific IgM antibody in as many as 20% of HIV/AIDS patients[11]. Several studies have suggested that detection of circulating antigen should be pursed for toxoplasma detection[22,23].

In this study diarrhea and toxoplasmosis were correlated with the absolute CD4 count ranges in which they occurred maximally (division according to the Centers for Disease Control and Prevention)[12]. Diarrheal cases have demonstrated a CD4 count between 200-499 cell/mm^3, whereas cases of toxoplasmosis have demonstrated below 200 cell/mm^3. This is consistent with data published in various studies[14,24-26]. This suggests that with progress in disease the CD4 count declines and the HIV infected patients become more prone to intestinal opportunistic infections.

In a developing country like India, the increasing prevalence of parasitic infections in HIV/AIDS patients raises the problem associated with the disease. Therefore it is suggested that simple steps like drinking safe water, maintaining high level of environmental and personal hygiene and avoiding contact with contaminated soil should be taken to prevent the occurrence of these diseases in AIDS patients. This study also suggests that parasitic infections vary with region and studies should be done in different regions to determine the current prevalence of intestinal parasitic infections in HIV infected patients. Early diagnosis and appropriate treatment of parasitic infections considerably contribute to longevity of HIV infected patients by delaying the disease progression.

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Conflict of interest statement

We declare that we have no conflict of interest.

Comments

Background

HIV infection has emerged as the leading cause of morbidity and mortality in many developed nations. The morbidity in these patients is mainly due to opportunistic infections caused by an immune system weakened by the virus. This study highlights the prevalent parasitic opportunistic infections in HIV positive patients of the Marathwada belt of the country where a large chunk of these patients reside. Many non-opportunistic infections were also reported in the study which caused prolonged and intractable diarrhea in these patients.

Research frontiers

Many intestinal pathogens were identified in this study. The prevalence of *Cryptosporidium* and *I. belli* induced diarrhea was found to be more than any other parasites. These findings are of significance as these intestinal pathogens can be easily eliminated by safe drinking water and a high level of environmental and personal hygiene. Thus, by identifying the various parasitic infections and using the above mentioned measures, morbidity in these patients can be drastically reduced.

Related reports

Seventy patients in this study were reported as harboring intestinal parasitic infections akin to studies by Pavie *et al*. However, although the predominant pathogen in this study was *Cryptosporidium parvum*, Pavie *et al.* have noted a predominance of *I. belli* in their study in France. This variation may be due to the geographic difference between the two studies. Toxoplasmosis was detected using a ImmunoComb IgM kit and a low prevalence of the disease was noted(2.4%). Authors advocate the use of antigen detection kits for the detection of toxoplasmosis. This seems the correct option as the antibody response in HIV may be low or even undetectable due to the weakened immune system.

Innovations & breakthroughs

This study provides the data about the common intestinal and other parasitic infections in HIV positive patients in this part of the country. The laboratories in this region may be strengthened to diagnose these parasites by common techniques like staining.

Applications

It is important to know the prevalent opportunistic parasites in the HIV positive patients in any region. This helps the clinicians to keep an eye on the development of

these diseases. It also helps the laboratories to think of the prevalent pathogens in any sample before going for the rare diagnoses.

Peer review

This is a good study in which the authors have noted the spectrum of opportunistic and other parasites among HIV positive patients. These infections were also correlated with the absolute CD4 count ranges in which they occurred maximally. Thus proving the data regarding the prevalent pathogens and the CD4 counts where they are expected to be present maximally, a keen lookout for these pathogens can be made. This will help to alleviate the morbidity of the HIV infected patients.

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