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Prevalence of Intestinal Parasites in HIV Seropositive Patients with and without Diarrhoea and its Correlation with CD4 Counts

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A B S T R A C T

Intestinal parasites are the most common cause of diarrhoea in HIV patients. The line of treatment being different for diverse parasites necessitates a definitive diagnosis of etiological agents. Thus the study was undertaken to detect enteric parasites in HIV infected patient with and without diarrhoea that were at different levels of immunity. The present study was conducted in between January 2013 - June 2014, at the Department of Microbiology, Santhiram General Hospital, Nandyal. A total of 100 HIV seropositive patients attending the Integrated Counselling and Testing Centre (ICTC), Santhiram General Hospital, Nandyal, Kurnool Dist, A.P was included in the study. These comprised of 50 HIV patients who presented with diarrhoea (study group) and 50 HIV patients without diarrhoea (control group). For each patient, demographic data including structured questionnaire was filled in the proforma. The CD4 cell counts were estimated by using the FACS count system. Out of 100 HIV infected patients, intestinal parasites were detected in 29% patients. Among all, *Cryptosporidium* appears to have the high prevalence (26%) followed by *Isospora* (10%), *Giardia* (6%), *Strongyloides* and *Entamoeba histolytica* (4%) each and *Cyclospora* (2%). In HIV infected patients with CD4 count < 200cells/ml. *Cryptosporidium* was the most common observed pathogen (26%). The maximum parasite isolation was in the patients whose CD4 cell counts were below 200 cells/ml. Parasitic infections were detected in 29% of HIV seropositive patients and various enteric parasites detected include Cryptosporidium (15), Isospora (05), Giardia intestinalis (04), Entamoeba histolytic (02) Cyclospora (01), Strongyloides (02).Routine screening of all HIV infected patients with low CD4 count for parasitic infections by using simple stool microscopic technique can help in easy diagnosis of approximate treatment and control of spread.

Introduction

Human immunodeficiency virus infection has become a global epidemic far extensive than what was predicted even a decade ago and the pace of the epidemic is accelerating in India (Anand *et al*., 2013). Since the beginning of the epidemic, estimates suggest

that more than 60 million people have become infected and more than 20 million people have died of HIV/AIDS, including 3 million deaths in 2001 alone of the global total number of people who are living with HIV, 95% live in developing countries. India, where 3.97 million Indians are living with HIV/AIDS, has the highest number of HIV infected persons in Asia (Anuradha, 2013).

In the coming years there is likely to be an increase in the number of HIV/Aids deaths, with worrying projections of 6.5 million deaths in 2030 and HIV/AIDS being the main burden of disease in some developing countries by 2015 (Arora *et al*., 2009).

In HIV infected patients, progressive decline in their immunological response makes them extremely susceptible to a variety of opportunistic infections. Opportunistic infections (O.I's) have been recognised as common complications of HIV infection. The spectrum of O.I's in the HIV infected patients varies from one region to another. Gastro intestinal infections are very common in patients with AIDS which presents commonly in diarrhea (Arora *et al*., 2006). Reports indicate that diarrhea occurs in 30-60% of AIDS patients in developed countries and in about 90% of AIDS patients in Haiti and Africa (Gupta, 2008).

Enteric protozoal infection is the commonest cause of diarrhea in HIV seropositive persons. Diarrhea was defined as passage of two or more liquid stools or three or more soft stools per day (Kumar, 2002). Diarrhoea associated with HIV infection may be acute or chronic. Acute diarrhoea is defined as diarrhoea of <14 days duration and chronic diarrhoea is defined as diarrhoea lasting for >14 days to four months or diarrhoea for more than a month. Diarrhoea occurs in almost 90% of patients with HIV in developing countries at sometime during the clinical course and is the present symptom of approximately a third of patients with HIV infection (Kumarasamy *et al*., 2005). Chronic diarrhoea is a major problem in HIV infected persons, affecting upto 76% of those with AIDS. It is associated with a 3.3 fold increase risk of disease progression. Chronic diarrhea significantly reduces the quality of life in patients with HIV infection and is an independent predictor of mortality in AIDS (Kumarasamy *et al*., 2005).

The common parasites causing AIDS associated with and without diarrhoea are (Mohandas *et al*., 2002)

Cryptosporidium parvum
Isospora belli
Cyclospora cayetanensis
Microsporidia
Strongyloides stercoralis
Entamoeba histolytica
Giardia lamblia

The outcome of the infection by enteric protozoan parasites is dependent on absolute CD4+ counts, with lower counts being associated with more severe disease and a greater risk of disseminated disease. CD4 counts of < 100 cells/µl poses greatest risk.

Materials and Methods

The study was conducted between January 2013 – June 2014, at the Dept of Microbiology, Santhiram Medical College, Nandyal.

The purpose of the study is to determine the prevalence of intestinal parasites and their association with diarrhoea in HIV infected patients. And to elucidate the association between intestinal parasitic infection and CD4 counts in HIV infected patients.

A total of 100 HIV seropositive patients attending the Integrated Counselling and Testing Centre (ICTC), Santhiram General Hospital, Nandyal, Kurnool Dist, A.P were included in the study. These comprised of 50 HIV patients who presented with diarrhoea (study group) and 50 HIV patients without diarrhoea (control group).

HIV infected patients were defined as those who had tested positive for HIV antibodies by three sequential rapid tests as per the recommendations given by the National AIDS Control Organization (NACO). All the tests were done after due patient consent and in accordance with the instituitional ethical guidelines.

For each patient, demographic data including a structured questionnaire was filled in the proforma.

Study group

Inclusion criteria

HIV seropositive patients with acute or chronic diarrhoea and

HIV seropositive patients who did not receive antiparasitic treatment for diarrhoea were included in the study

Exclusion criteria

HIV seropositive patients who received antiparasitic treatment for diarrhoea in the past 14 days were excluded.

Parasitological examinations

Stool samples were collected in a wide mouthed, leakproof, clean, dry, plastic containers. The patients were instructed to avoid contamination of stool with urine or water. Fresh stool samples were collected from each subject and were examined for parasites as per the standard laboratory procedures immediately. If there was a delay in processing the samples, they were preserved at 4°C. A small portion of sample was emulsified in a drop of saline and Lugol's iodine on the slide and observed under microscope. The samples were concentrated by formol ether sedimentation and Sheather's sugar floatation technique. Thereafter the samples were stained by Modified acid fast technique, Auramine-O staining technique for *C.parvum*, *I.belli*, *Cyclospora* and Modified Trichrome stain for Microsporidia.

Results and Discussion

A total of 100 stool samples from HIV seropositive patients were examined for the enteric parasitic infection. Out of 100 HIV infected patients, 58 (58%) were male patients and 42 (42%) were female patients. The majority of HIV infected patients belonged to 31 – 40 year age group in males and 20 – 30 year age group in females. Intestinal parasites were detected in all the age groups and in both the sexes.

Cryptosporidium was the most common parasite detected in 13/50 (26%) patients with diarrhoea. *Isospora* was next commonly isolated parasite 5/50 (10%).

Majority of the parasites were detected in patients with CD4 counts < 200 cells/ml. Out of 21 stool samples positive for coccidian parasites, all 21 were detected by Sheather's sugar floatation method and only 17 were detected by Formol-ether concentration method.

In the present study, a total of 100 HIV seropositive patients were studied. Majority of the patients (36%) belonged to age group 31-40 years. Similar age group studies have been reported in HIV seropositive patients

by others (Vyas *et al.,* 2012; Uppal *et al.,* 2009).

In the present study, intestinal parasites were detected in 58% of HIV infected patients with diarrhoea and without diarrhoea. This study corroborates well with other studies which reported a prevalence of intestinal parasites ranging from 30% to 60%in HIV infected patients (Mukhopadhya *et al.,* 1999; Vyas *et al.,* 2012).

In the present study, coccidian parasites were detected in 73.14% of stool samples of HIV infected patients. Other studies reported a prevalence rate of 76.3% (Gupta *et al.,* 2008)

In the present study, *Cryptosporidium* was found to be predominant (26%) intestinal parasite associated with diarrhoea which is in accordance with other studies (Basak *et al.,* 2010; Mohan Das *et al.,* 2002) and the association of Cryptosporidium with and without diarrhoea in HIV infected patients is highly significant ($p = 0.000$ i.e < 0.01).

Isospora was detected in 18% (9 out of 50) of HIV positive diarrhoea patients, slightly lower detection rates as compared to earlier studies may be due to intermittent shedding of oocysts.

In the present study, *Cyclospora* was detected from stools of 2% (1/50) HIV positive diarrhoea patients. Cyclospora was detected in 0.98% and 4.9% of HIV positive diarrhoea patients in previous studies done in Southern India (Kumar *et al.,* 2002; Gupta *et al.,* 2008).

Table.1 Distribution of age and sex among 100 HIV infected patients

Age (years)	**Males n=58**	**Females n=42**
20 – 30	10	24
31 – 40	24	12
41 – 50	14	5
51 – 60	8	1
61 – 70	2	-

Table.2 Enteric parasites detected from HIV infected patients with and without diarrhea

Parasites	**No.of parasites in patients with diarrhoea n=50**	**No.of parasites in patients without diarrhoea n=50**
Cryptosporidium	13	2
Isospora belli	5	-
Giardia lamblia	3	1
Entamoeba histolytica	1	1
Cyclospora cayetanensis	1	-
Strongyloides	2	-

Table.3 Comparative results of Formol-ether concentration method and Sheather's sugar floatation method for coccidian parasites

Total no.of stool samples positive for parasites	No.of positives by Formol-ether concentration method	No.of positives by Sheather's sugar floatation method
21	17	21

Table.4 Associaion between parasites isolated and CD4 counts of HIV infected patients

Parasites	CD4counts < 200 cells/µl	CD4 counts 200 – 500 cells/µl	CD4 counts >500 cells/µl
Cryptosporidium	10	3	2
Isospora belli	5		
Giardia lamblia	2	1	1
Entamoeba histolytica	1	1	
Cyclospora cayetanensis	1	-	-
Strongyloides	2		
Total	21	5	3

Microsporidia was not detected in the present study similar to a few other studies because of striking geographical variations in the prevalence of the parasite in HIV infected patients.

Giardia lamblia and *Entamoeba histolytica* were the common non-coccidian protozoan parasites demonstrated in the stool of HIV infected patients. Detection of *Giardia* (4%) and *Entamoeba histolytica* (2%) in the present study is consistent with the findings of other authors (Talib and Singh, 1998; Mukhopadhya *et al.,* 1999).

It is concluded from the present study that Cryptosporidium is the commonest intestinal protozoan parasite in HIV infected patients with diarrhoea. Hence it is necessary that HIV/AIDS patients presenting with diarrhoea should be screened for enteric parasites with most accurate staining methods. Based on the present study we conclude that special staining methods like Modified Z-N method and concentration methods like Sheather's sugar floatation method should be employed as a part of routine testing for detection of intestinal parasites in HIV infected patients. This will guide clinicians to start an early and appropriate treatment and thus improve the quality of life of such patients. Routine screening of all the HIV infected patients with low CD4 counts for coccidian parasites by using simple stool microscopic techniques can help in early diagnosis, initiation of appropriate treatment and control of spread.

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