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OPPORTUNISTIC INTESTINAL PARASITIC INFECTIONS IN HIV/AIDS PATIENTS PRESENTING WITH DIARRHEA AND THEIR CORRELATION WITH CD4+ T-LYMPHOCYTE COUNTS

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ABSTRACT

Background: *The objective of this study was to determine the prevalence of opportunistic intestinal parasitic infections in HIV/AIDS infected individuals with special emphasis on Cryptosporidium, Isospora, and Cyclospora, as well as their association with diarrhea at different levels of immunity.* ***Methods:*** *The study period was from January 2010 to November 2010. Stool samples were collected from 359 HIV positive patients with diarrhea attending the ART centre and integrated counseling and testing centre (ICTC) of R.I.M.S, Raichur. Simultaneously, CD4 counts were recorded to assess the status of HIV infection vis-à-vis parasitic infection. The identification of pathogens was done on the basis of direct microscopy and staining technique.* ***Results:*** *Out of the 359 HIV positive patients with diarrhea, enteric parasites were isolated from 195(54.31%) of HIV positive patients with diarrhea compared to 12(12%) of HIV positive patients without diarrhea. Cryptosporidium parasite (15.87%) was the predominant parasite isolated in HIV positive patients with diarrhea followed by Isospora belli (10.02%) and Cyclospora sp (5.84%). The maximum parasitic isolation was in the patients with CD4+ T cell counts < 200 cells/µl.* ***Conclusion:*** *Immunodeficiency increased the risk of having opportunistic parasites and diarrhea. This study shows CD4+ T cell count <200 cells/µl and diarrhea has high probability of association with opportunistic parasitic infections. Regular monitoring of CD4 counts and screening of these opportunistic agents in the HIV infected patients with diarrhea will help to reduce the mortality and morbidity with administration of appropriate therapy.*

KEYWORDS

HIV sero-positive patients;CD4+ T cell counts;Opportunistic intestinal parasites; Cryptosporidium parvum;Isospora belli;

INTRODUCTION

Human immunodeficiency virus (HIV) infection, a worldwide phenomenon, is a serious public health problem.[1] Reports indicate that diarrhea occurs in 30-60% of AIDS patients in developed countries and in about 90% of AIDS patients in developing countries.[2] A decrease in CD4+ T-lymphocyte counts is responsible for the

profound immunodeficiencies that lead to various opportunistic infections in HIV infected patients.[3] Intestinal infection causing diarrheal diseases is a dominant contributor to high death rates and developmental retardation in developing countries.[4] Recently there has been great interest in the impact of HIV on the gut[5], both because of virally induced T cell depletion [6-8], and because intestinal damage may drive bacterial translocation and hence immune activation.[9]

The presence of opportunistic parasites like Cryptosporidium parvum, Cyclospora cayetanensis, Isospora belli, and Microsporidium sp is documented in patients with AIDS.[10] The incidence and prevalence of infection with a particular enteric parasite in HIV/AIDS patients is likely to depend upon the endemicity of that particular parasite in the community.[11] As most of the protozoan infections are treatable, it is important that an early and accurate diagnosis be made.[12]

The present study was undertaken to study the prevalence of opportunistic intestinal parasitic infections in HIV/AIDS patients presenting with diarrhea and their correlation with CD4+ T-lymphocyte counts, because of paucity of data on correlation of CD4+ T- cell counts and the etiology of diarrhea among HIV patients in this part of India.

MATERIALS AND METHODS

The study was conducted from January 2010 to November 2010 in department of Microbiology, RIMS (Raichur Institute of Medical Sciences), Raichur. Samples were collected from ART and ICTC centre, RIMS hospital, RIMS, Raichur. The study group included 359 HIV-positive patients presenting with diarrhea and control group having 100 HIV-positive patients without diarrhea. Study patients were interviewed using the structured questionnaire. Information was obtained on demographic characteristics, present and past history of diarrhea and anti-parasitic treatment. Diarrhea was defined as two or more liquid or three or more soft stools per day. Patients who received anti-parasitic treatment for diarrhea in the past 14 days were excluded from the study. Blood samples (plain and EDTA) 5ml each were obtained from study group. Serum samples were used for HIV testing. HIV sero status of the patients was determined by using commercially available ELISA antibody tests (Genetic system, Bio-Rad Labs, USA and Tridot, J Mitra & Co, New Delhi) using National AIDS Control Organization (NACO) recommended algorithm.[13] CD4 cell counts were measured by using a FACS count system (Becton Dickinson, Singapore BD). Patients were categorized by their immune status according to the 1993-revised classification system for the HIV infection by CD4 T- cell categories.[14]

Stool examination:

Stool samples were collected in labeled, leak proof, clean sterile plastic containers and were transported to the laboratory within three hours of collection. 10% formal saline was used to preserve stool samples. According to the WHO standard stool samples were examined microscopically following direct and formalin-ether concentration method.[15] The consistency of stool samples was noted. A direct wet mount of stool samples in normal saline (0.85%) was prepared and examined for the presence of motile intestinal parasites and trophozoites under light microscope. Lugols iodine staining was used to detect cysts of intestinal parasites. The modified acid fast staining technique was used for coccidian parasites (Cryptosporidium, Cyclospora, and Isospora) after concentration technique.[16]

Data analysis:

Statistical analysis was done by using Epi Info version 3.5.1. Strength of association was

measured by using the Chi-square test and its associated P value. Values were considered to be statistically significant when the P value was less or equal to 0.05.

RESULTS

In the present study out of 359 HIV positive patients with diarrhea, 54.31% (195/359) of patients showed enteric parasitic infection. Cryptosporidium was the most common parasite isolated in 57 (15.87%), followed by Isospora 36 (10.02%) and Cyclospora 21 (5.84%) in HIV-positive patients with diarrhea respectively. Other parasites like Entamoeba histolytica 35 (9.74%), Ascaris lumbricoides 19 (5.29%), Ancylostoma duodenale 11 (3.06%), Taenia sp 09 (2.5%), and Strongyloides stercoralis 07 (1.94%) were isolated in HIV-positive patients with diarrhea respectively. 12% (12/100) of Enteric parasites were detected in controls (i.e. 100 HIV positive patients without diarrhea), of which Cryptosporidium 03 (3%), E. histolytica 02 (2%), A. lumbricoides 03 (3%), and A. duodenale 04 (4%) respectively [**Table 1**].

Table 1: Enteric parasites detected from HIV sero-positive patients with and without diarrhea.

Parasites found	No of parasites in HIV patients with diarrhea, N=359	No of parasites in HIV patients without diarrhea, N=100
Cryptosporidium sp	57(15.87%)	03(3%)
Isospora sp	36(10.02%)	00(0%)
Cyclospora sp	21(05.84%)	00(0%)
E. histolytica	35(09.74%)	02(2%)
A. lumbricoides	19(05.29%)	03(3%)
A. duodenale	11(03.06%)	04(4%)
Taenia sp	09(02.50%)	00(0%)
S. stercoralis	07(01.94%)	00(0%)
Total	195(54.31%)	12(12%)

X^2 (Chi square) test value = 3.88 with degree of freedom 1

P = 0.04 which is <0.05 (result significant) for enteric parasites isolated in HIV positive patients with and without diarrhea.

In 127 HIV-positive patients with diarrhea having CD4+ T cells count <200 cells/µl, 85.82% of patients were positive for enteric parasites. While 65 HIV-positive patients with diarrhea having CD4+ T cells count in the range of 200-500 cells/µl, 63.07% of patients were positive for enteric parasites and 167 HIV-positive patients with diarrhea having CD4+ T cells count >500 cells/µl, 26.94% of patients were positive for enteric parasites respectively. Opportunistic intestinal parasites were detected in 66.14% in HIV-positive patients with diarrhea having CD4+ T cells count <200 cells/µl, Cryptosporidium (47) was the most common pathogen followed by Isospora (26) and Cyclospora (11). While in HIV-positive patients with diarrhea having CD4+ T cells count ≥200 cells/µl, opportunistic intestinal parasites were detected in 12.96% (Cryptosporidium, Isospora, and Cyclospora were seen in 10 patients each) [**Table 2**].

Table 2: Association between parasites isolated and CD4+ T-cell counts of HIV positive patients with diarrhea.

Parasites found	CD4+ T cell count <200 cells/µl, N=127	CD4+ T cell count 200-500 cells/µl, N=65	CD4+ T cell count >500 cells/µl, N=167
Cryptosporidium sp	47(37.00%)	07(10.76%)	03(01.79%)
Isospora sp	26(20.47%)	07(10.76%)	03(01.79%)
Cyclospora sp	11(08.66%)	07(10.76%)	03(01.79%)
E. histolytica	10(07.87%)	14(21.53%)	11(06.58%)
A. lumbricoides	09(07.08%)	03(04.61%)	07(04.19%)
A. duodenale	00(00.00%)	00(00.00%)	11(06.58%)
Taenia sp	02(01.57%)	00(00.00%)	07(04.19%)
S. stercoralis	04(03.14%)	03(04.61%)	00(00.00%)
Total	109(85.82%)	41(63.07%)	45(26.94%)

X^2 (Chi square) test value = 35.22 with degree of freedom 1

P = 0.001 which is <0.05 (result highly significant) for enteric parasites in HIV positive patients with diarrhea having CD4+ T cell count <200 cells/µl and CD4+ T cell count ≥200 cells/µl.

X^2 (Chi square) test value = 7.22 with degree of freedom 2

P = 0.02 which is <0.05 (result significant) for opportunistic parasites in HIV positive patients with diarrhea having CD4+ T cell count <200 cells/µl and CD4+ T cell count ≥200 cells/µl.

DISCUSSION

Decline in the immunological and the mucosal defensive mechanism predisposes the HIV positive individuals to gastrointestinal infections. Most of the morbidity and mortality in such patients is due to opportunistic infections.[17]

This study reports the prevalence of opportunistic and common intestinal parasitic infection in HIV-positive patients with and without diarrhea.

Diarrhea is one of the most common manifestations in HIV patients and is strongly associated with low CD4+ cell counts. Various studies have shown a rise in diarrhea caused by parasites which includes both opportunistic agents causing severe form and non-opportunistic agents causing treatable diarrheal illness.[18] We compared the prevalence of these parasites in HIV-positive patients with and without diarrhea (54.31% compared to 12% in patients with diarrhea and without diarrhea respectively P<0.05) [**Table 1**]. This study corroborates well with other studies which reported a prevalence of intestinal parasites, ranging from 30% to 60% in the HIV-positive diarrhea patients.[19-22] Studies by other workers have reported rates of 39%-72.6% in diarrheal and 12.9%-23.4% in non-diarrheal cases.[23-25]

Cryptosporidium was found to be predominant 15.87 %(57/359) enteric parasite associated with HIV-positive patients with diarrhea in this study. Earlier studies done in southern India have noted isolation of Cryptosporidium between 10% and 19% in HIV-positive diarrhea patients.[20,22,26,27] In India, there have been reports from the mid-1990s on the prevalence of symptomatic Cryptosporidiosis in HIV infected adults from different parts of the country, ranging from as low as 0.7% to as high as 81%. A high prevalence of 80% was reported from a study in Imphal[28] and another in Maharashtra [29] but both had very small sample sizes. However, the data on prevalence are highly varied and could reflect geographical differences, as well as differences in the populations, socioeconomic status and access to potable water.

In present study, Isospora was isolated from 10.02% of HIV-positive patients with diarrhea, which was similar to the previous study conducted in Jaipur, India recently.[30] Little higher percentage of Isospora were isolated with studies made by Ramana et al (17.1%)[31], Nazeema et al (17.1%)[32], Satheesh et al (18.6%).[23] While some studies reported higher prevalence of Isospora belli then Cryptosporidium sp. [33, 34] In this study slightly lower isolation rate as compared to earlier studies may be due to asymptomatic shedding of oocysts and treatment with trimethoprim-sulphamethoxazole for other infections in AIDS cases.

In our study, Cyclospora was isolated from 5.84% of HIV-positive patients with diarrhea. Cyclospora was isolated in 0.98% [21], 4.9% [22], and 24% [19] of HIV-positive patients with diarrhea in previous studies done in southern India. Also 8.5% [32] and 8.6% [30] of Cyclospora was isolated from HIV positive patients with diarrhea. Other non-opportunistic parasites detected in this study were E. histolytica (9.74%), A. lumbricoides (5.29%), A. duodenale (3.06%), Taenia sp (2.5%), and S. stercoralis (1.94%). The reported prevalence of non-opportunistic parasites varied from 5-30 percent in HIV-positive patients.[35 36] In the present study non-opportunistic parasites were detected in 22.56% HIV positive patients with diarrhea across different CD4 groups, thus highlighting the need for early detection and treatment of such infections to reduce the morbidity in HIV-positive patients with diarrhea.

In present study among 195 enteric parasites isolated, 55.89% patients were positive for enteric parasites with CD4+ T cell counts <200 cells/µl, while 21.02% patients were positive for enteric parasites have CD4+ T cell counts in the range between 200-500 cells/µl, and 23.07% patients were positive for enteric parasites having CD4+ T cell counts >500 cells/µl. Patients with CD4+ T cell counts <200 cells/µl, enteric parasites could be identified in 85.82% (109/127), and opportunistic parasites were detected in as many as 66.14% (84/127) of patients [i.e. Cryptosporidium was most common pathogen in 47 patients, Isospora in 26 patients, and Cyclospora in 11 patients]. Of the 232 patients with CD4+ T cell counts ≥200 cells/µl, enteric parasites could be identified in 37.06%, and opportunistic parasites were detected in as many as 12.93% (30/232) of patients [i.e. Cryptosporidium, Isospora, and Cyclospora in 10 patients each respectively].

It was observed that the CD4 cell count influenced the cause of diarrhea as well as the diagnostic yield.[37] In agreement with previous studies those parasites associated with HIV were encountered more often as the CD4+ T cell count fell below 200/µl. [38, 39] Immunodeficient state makes them more susceptible to these infections and once established they are not able to prevent the proliferation or clear the infecting agent.[40] Cryptosporidium infection is an important cause of diarrhea in the developing world.[41] The correlation between the decreased no of CD4+ T cells and the risk of Cryptosporidium infection is evidence of the critical role that immune cells play in immunity.[42]

CONCLUSION

Infection of Cryptosporidium, Isospora, and Cyclospora was significantly higher among HIV-positive patients with diarrhea, particularly in those with <200 CD4+ T cell count. Our findings highlighted the importance of screening the HIV sero-positive individuals and evaluating their absolute CD4 counts regularly. As the intestinal parasitic infections vary with the geographical areas, the studies must be aimed out in different regions, to evaluate the frequent causes of the intestinal parasitic infections in HIV sero-positive patients. This highlights the need for early

detection and treatment of such infections among the HIV infected patients, to reduce the morbidity and the mortality.

Limitations:

There are some limitations of our study. It is possible that some parasites were not detected in this study because methods for Microsporidium detection, adhesive tape or anal swab for Enterobius vermicularis were not used. Therefore, the prevalence of other intestinal parasites among the study participants may have been underestimated.

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