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Research Article

Acid fast cysts in diarrheal stool samples of HIV positive patients

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ABSTRACT

Background: Gastrointestinal infections are very common in patients with HIV infection or AIDS, and diarrhea is a common clinical presentation of these infections. Acid fast protozoans are very commonly responsible for diarrhea in HIV positive patients leading to death in many cases.
Methods: The study group included 50 HIV seropositive patients suffering from diarrhea and the control group included 50 HIV seronegative patients suffering from diarrhea. The stool samples collected were concentrated using formol-ether concentration technique and stained using modified Ziehl-Neelsen's staining procedure.
Results: Among the diarrheal stool samples of HIV positive patients (n=50), 17 (34%) were positive for acid fast cysts, and among the HIV negative stool samples (n=50), 2 (4%) were positive for acid fast cysts. Cryptosporidium oocysts were detected in 15 (30%) and Isospora oocysts in 2 (4%) of the samples in the study group. Cryptosporidium oocysts were detected in 2 (4%) of the samples in the control group. There existed a significant difference between the positivity of HIV-positive and HIV-negative diarrheal stool samples.
Conclusion: Timely and effective diagnosis could help in delivering appropriate treatment in an already immunocompromised patient.

Keywords: HIV, Gastrointestinal infections, Acid fasts cysts, Modified acid fast stain, Cryptosporidium, Isospora

INTRODUCTION

Gastrointestinal infections are very common in patients with HIV infection or AIDS, and diarrhea is a common clinical presentation. Reports indicate that diarrhea occurs in 30-60% of AIDS patients in developed countries, and in about 90% of AIDS patients in developing countries.[1] The etiology for such diarrhea could be either parasitic, bacterial, fungal, enteric virus; or HIV itself might contribute. Several species of protozoa have been associated with acute and chronic diarrhea in HIV disease. These include: acid fast protozoans, Giardia intestinalis, Entamoeba histolytica, Cyclospora species, Blastocystis hominis, and Dientamoeba fragilis, but convincing evidence is lacking as to the causality of the last two protozoans. Besides these, the nematode Strongyloides stercoralis, a ubiquitous parasite in tropical and subtropical areas, can cause diarrhea and overwhelming infestation (hyperinfection syndrome) in patients with a variety of immunosuppressive disorders, including HIV/AIDS.[2]

The acid fast protozoans mainly responsible for diarrhea in HIV positive patients include Cryptosporidium, Cyclospora, Isospora and Microsporidia. Many HIV positive patients have died because of diarrhea caused by them, and detecting them in the stools is an AIDS defining criteria. Infections caused by them cannot be differentiated unless specific stool examination procedures are done. Stool examination by modified acid

fast staining is a reliable method for the diagnosis of these infections which helps in timely diagnosis and in treatment of such individuals. With the above back ground, the present study was done to identify the acid fast cysts in diarrheal stool samples of HIV positive and HIV negative patients, and to compare them with the other reported similar studies.

METHODS

Study design: Prospective and comparative study.

The study group included 50 known HIV seropositive patients with complaints of diarrhea attending to the outpatient and inpatient departments. Stool samples were collected after taking an informed consent. The control group included 50 known HIV seronegative patients suffering from diarrhea in the outpatient and inpatient departments. Persons who had received antibiotics within the previous 2 weeks were excluded from the study. Stool samples were collected taking standard precautions in wide mouthed containers, and were processed in the lab.

The stool samples were concentrated using Formol- Ether concentration method[3] and the deposit was smeared on a slide, fixed with methanol and stained using modified Ziehl- Neelsen's staining procedure using 1% sulfuric acid as the decolorizer.[4] The stained smear was screened for acid fast cysts and their structural details observed.

RESULTS

Among the diarrheal stool samples of HIV positive patients (n=50), 17 (34%) were positive for acid fast cysts, and among the HIV negative stool samples (n=50), 2 (4%) were positive for acid fast cysts.

Table 1: Identification details of the cysts from test and control groups.

Diarrheal stool samples	Cryptosporidium parvum	Isospora belli	Other acid fast cysts	Total
HIV positive (n=50)	15	02	Nil	17 (34%)
HIV negative (n=50)	02	Nil	Nil	2 (4%)

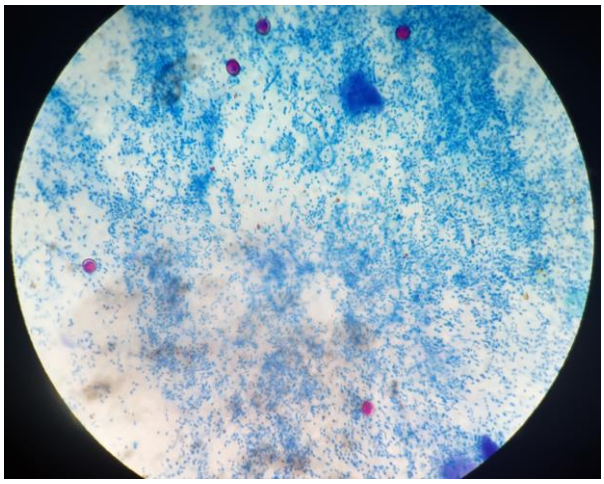

Figure 1: Smear showing acid fast cysts under oil immersion lens.

Applying the test of significance for difference of proportion, the calculated value being 4.138, was found to be greater than the critical value at 5% level of significance. The null hypothesis got rejected, indicating significant difference between the two groups.

DISCUSSION

Intestinal protozoal parasitic infections are the commonest and major cause of morbidity and mortality in HIV positive cases worldwide. These organisms usually cause a self-limiting illness in immunocompetent individuals, but in the case of immunocompromised patients they can cause life threatening, profuse watery diarrhea.[5] HIV infection is a major threat to public health in India. Numerous opportunistic infections occur due to deterioration of the immune system because of HIV infection, of which intestinal parasitic infections are a universally recognized problem. They largely present with diarrheal symptoms leading to life threatening complications.[6] Cryptosporidium is a well-established cause of diarrhoea among HIV infected patients worldwide with prevalence of infection ranging from 3% in the developed countries to 50% in the developing countries. The mechanisms by which Cryptosporidium causes diarrhoea is not well known, inflammatory response to the infection is variable and may be modified by co-pathogens such as Cytomegalovirus.[7] The infestation of the gastro-intestinal tract by coccidian parasites is kept in check in the immunocompetent people by the host immune system. The circulating antibodies reduce the severity of symptomatic infections. The cell mediated immunity in cooperation with antibody mediated immunity helps in resolving the infection. As the immunity is compromised in HIV positive persons, there is a high prevalence of these infestations. Also, the severity of the infection is increased in these patients and may lead to debilitating illness.[4] Thus the coccidians have a greater significance in producing severe disease in the immunocompromised group. Many physicians remain unaware of their clinical importance.[8] It would be advisable to recognize that cryptosporidiosis can present

with just chronic weight loss and other nonspecific symptoms devoid of diarrhea. Hence, a higher index of suspicion for clinical cryptosporidiosis in HIV patients, including those with chronic weight loss with or without diarrhea, is recommended. Additionally, laboratory testing for Cryptosporidium in HIV infected patients is highly recommended in order to have a better understanding of the epidemiology and management.[9] Comparative studies rank Kinyoun Cold Acid Fast (KCAF) staining method highest in terms of the reagent cost, hands-on time required, yield, ease of handling and ability to process large numbers of specimens.[10] KCAF staining method was used for identification of the acid fast cysts in the present study also. The present study was conducted to compare the positivity of acid fast cysts in HIV positive diarrheal stool samples (n=50) with that of HIV negative diarrheal stool samples (n=50). Coccidian parasites (acid fast) were detected in 17/50 (34%) of the diarrheal stool samples from HIV positive patients in the present study, while Gupta et al.[6] and Amatya et al.[11] had reported coccidian parasites in 14% and 52.8% stool samples from HIV positive patients in their respective studies. Cryptosporidium oocysts were detected in 15 (30%) and Isospora oocysts in 2 (4%) of the samples in the present study. Nautiyal et al.[10] had reported Cryptosporidium oocysts in 17.5% of their HIV positive diarrheal stool samples. Kumurya et al.[7] had reported Cryptosporidium oocysts only in 6% of their HIV positive diarrheal stool samples. Acquah et al.[12] had reported Cryptosporidium oocysts, with no Isospora detection from 33.3% of HIV positive diarrheal stool samples in their study. Kulkarni et al.[1] had reported Cryptosporidium oocysts in 12% of HIV positive diarrheal stool samples, but also reported detection of other acid fast cysts like Isospora - 8%, Microsporidia - 1% and Cyclospora - 0.7% in their study. In contrast to all the above studies, Ashihabegum et al (13) had reported detection of only Cyclospora oocysts (24%) in their study on HIV positive stool samples while screening for acid fast coocidian parasites. Agnes Kurniawan et al.[14] had done PCR to screen HIV positive chronic diarrhoeal stool samples for Cryptosporidium species and reported the incidence to be 12.89% in their study. Joshi et al.[15] had reported Cryptosporidium oocysts in 12% of HIV positive stool samples. Gupta et al.[6] had screened HIV positive samples for acid fasts cysts and reported the incidence to be 14%; with a higher incidence of Isospora oocysts (23.2%) and lower incidence of Cryptosporidium oocysts (9.3%) detection. Shrihari et al.[5] had reported identification of Cryptosporidium oocysts in 26.92% of HIV positive diarrheal stool samples. The present study (34%) correlates well with Shrihari et al. - 26.92% and Acquah et al. - 33.3% for identification of Cryptosporidium oocysts. Beula et al.[16] had done a study to screen diarrheal stool samples, with an unknown HIV status, and reported a very high incidence of detection of Cryptosporidium oocysts - 71.42%. In a similar study done by Newman et al.,[17] these authors also had reported a high incidence of Cryptosporidium oocysts (63.2%) in diarrheal stool samples with HIV unknown status. Newman et al.[17] had also reported an incidence of Cryptosporidium oocysts in 6.8% in stool samples without diarrhea and with unknown HIV status in their control group. Cryptosporidium oocysts were reported in 2 (4%) in diarrheal stool samples from HIV negative patients in the present study. Other reported studies include Nautiyal et al.[10] - 4.5%, Gupta et al.[6] - 2% of coccidians in HIV negative stool samples, and Kumurya et al.[7] had reported nil detection of coccidian parasites in HIV negative stool samples with diarrhea. Isospora oocysts were detected in 2 samples (4%) from HIV positive patients with diarrhea in the present study. Other reported studies include Kulkarni et al.[1] - 8% in HIV positive patients with diarrhea, Acquah et al.[12] - nil detection of Isospora oocysts in their study on HIV positive stool samples with diarrhea, all the acid fast cysts they detected were Cryptosporidium oocysts only (33.3%). Gupta et al.[6] had reported a high detection rate of Isospora oocysts (23.2%) in stool samples from HIV positive persons. A high prevalence of intestinal parasitic infections in HIV positive individuals could be due to contaminated water supply and lack of personal hygiene, which is common in a rural scenario.[5] But timely diagnosis is hampered by the delay in the diagnosis of HIV and the lack of resources for its investigation in rural parts of developing countries. Patients from the rural areas belonging to a poor socio-economic background cannot afford diagnosis and treatment. Along with early diagnosis and treatment, other steps also should be taken to improve the water supply, sanitation and to provide health education to prevent the morbidity and mortality associated with HIV infected individuals.[5] The present study brings out the importance of having a high index of suspicion and screening of stool samples from HIV positive patients for acid fast coccidian parasites routinely to deliver timely diagnosis and helping in giving appropriate and timely treatment to the ailing patients. The present study has limitations like only a single stool specimen was examined, and more sensitive techniques like PCR have not been used as done by Agnes Kurniawan et al.;[14] and thus the prevalence of the infection may have been under estimated.

CONCLUSION

50 diarrheal stool samples from HIV positive patients and 50 diarrheal stool samples from HIV negative patients were concentrated using formol-ether concentration method and stained using modified Ziehl-Neelsen's staining procedure using 1% sulfuric acid as the decolorizer. Among the diarrheal stool samples of HIV positive patients (n=50), 17 (34%) were positive for acid fast cysts, and among the HIV negative stool samples (n=50), 2 (4%) were positive for acid fast cysts. Cryptosporidium oocysts were detected in 15 (30%) and Isospora oocysts in 2 (4%) of the samples in the present study. Cryptosporidium oocysts were reported in 2 (4%) in diarrheal stool samples from HIV negative patients in the present study. There existed a significant difference between the positivity of HIV-positive and HIV-negative

diarrheal stool samples. Routine screening of stool samples from HIV positive patients could help in delivering appropriate treatment in an already immunocompromised patient.

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