Research Paper

VOLUME - 3 | ISSUE - 2 | April - 2019 | ISSN (O) : 2663-2888 | ISSN (P) : 2663-287X

A STUDY OF STOOL SAMPLE FROM HIV POSITIVE AND HIV NEGATIVE AT ANDHRA PRADESH

Gundala Obulesu PHD Research Scholar, Department Of Microbiology, Bharath University, Chennai, India.

Dr. A. R. Hanumanthappa Professor, DEPT. Of Microbiology, J.J.M. Medical College, Davnagere. India.

Dr E. Prabhakar Reddy Professor, Department Of Biochemistry, Sree Lakshmi Narayana Institute Of Medical Sciences, Bharath Univarsity, Pondicherry, India.

ABSTRACT

BACKGROUND : To investigate the variations, if any, in the types of intestinal parasites in HIV positive and HIV negative individuals.

MATERIALS AND METHODS: for their HIV status in our laboratory by ELISA and TRIDOT, sample stool,Macroscopic and Microscopic examination,Formal – ether concentration technique f, Saline wet mount, Iodine wet mount, Modified acid-fast stain.

RESULTS : For the purpose of statistical analysis, the enteric parasites are divided into the coccidian group (Cryptosporidium+Isospora belli) and conventional enteric parasites (E.histolytica, G.intestinalis and A.lumbricoides),Enteric parasitic infection is more common in HIV positive patients with diarrhea than HIV positive patient without diarrhea or HIV negative individuals.

CONCLUSION: The study period of one year and eight months revealed that it is difficult to locate / identify a HIV positive person in a place like Kadapa on account of confidentiality / lack of awareness among the population.Detection of intestinal parasites is 17.3% in HIV positive patients and 12% in HIV negative individuals.

KEYWORDS

AIDS, HIV, Parasite.

ARTICLE HISTORY

Submitted : 22 January 2019 | **Accepted : 19 March 2019** | **Published : 05 April 2019**

***Corresponding Author** Gundala Obulesu

PHD Research Scholar, Department Of Microbiology, Bharath University, Chennai, India. obulesu100@gmail.com

INTRODUCTION:

Since the original description of AIDS (Acquired Immunodeficiency Syndrome) in 1981 by Michael Gottlieb and his colleagues in Losangeles, USA, a wide variety of gastrointestinal manifestations mainly opportunistic enteric parasitic infestations are described in patients with HIV infection [1]. Diarrhea is a common clinical manifestation of these infections. Identification of the etiological agent of diarrhea in HIV patient is very important as it can help in institution of appropriate therapy and reduction of morbidity and mortality [2].

It has been estimated that 30 to 50 % of patients with AIDS in the USA and about 90% in Africa and Haiti suffer from chronic diarrhea [3]. Infection caused by different pathogens cannot be differentiated clinically unless specific fecal examination is carried out. The isolation rates of intestinal parasites in patients with AIDS and chronic diarrhea vary from 40% to 83% and the parasitic agents also differ markedly from region to region [3].Until AIDS epidemic, several of the presently recognized parasitic infections were almost unknown as cause of human diseases[1].

Diarrhea is the presenting symptom of approximately a third of patients with HIV infection. Chronic diarrhea significantly reduces the quality of life in patients with HIV infection and is an independent predictor of mortality in AIDS. A variety of enteric pathogens have been isolated from AIDS patients with diarrhea but it is not clear that these enteric infections are necessarily associated with the presence of diarrhea [4]. The diarrhea wasting syndrome in association with a positive HIV serology test, is an AIDS-defining illness in the World Health Organization (WHO)'s classification [5]. Cryptosporidium and Isospora belli, two of the intestinal coccidian parasites known to be the causative agents of diarrhea in animals, have now emerged as one of the main causes of prolonged life threatening diarrhea in immune compromised hosts particularly so in patients with AIDS[6]. Intestinal microsporidiosis and cyclosporiasis have also been reported with increasing frequency in HIV infected patients[7] In HIV infected patients, progressive decline in their immunological responses makes them extremely susceptible to a variety of common and opportunistic infections.

MATERIALS AND METHODS:

The present study was conducted from july 2014 to December 2016FIMS kadapa.

STUDY GROUP:

Seventy-five HIV reactive (positive) patients (age 15 to 59 years) were identified with the help of clinicians of RIMS and FIMS. These patients were being treated by the clinicians for recurring ailments. The study group comprised of 55 male and 20 female HIV positive patients.

CONTROL GROUP:

Fifty HIV non-reactive individuals (34 male and 16 female), aged 18 to 60 years, admitted in RIMS and FIMS, kadapa for various ailments in surgical wards were included in the study as control group. stool samples were collected from these patients after collecting relevant information such as age, sex, occupation, present complaint, whether on ART (antiretroviral therapy), and treatment given etc. Both diarrheic and non-diarrheic stool samples were collected for parasitological examination.

Total number of fecal samples examined = 125

Laboratory diagnosis of HIV infection:

The HIV positive cases included in this study were identified through the clinicians, who were treating them for their recurrent ailments. They were investigated and identified as HIV reactive by serological tests done elsewhere. All of the patients were reported ELISA positive

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(as per WHO strategy-II 1993) for HIV-1/HIV-2 with at least two different kits [1].

The individuals included under the control group were screened for their HIV status in our laboratory by ELISA and TRIDOT (J. Mitra and co. Ltd, New Delhi) [7].Method during admission into surgical wards at RIMS and FIMs, during July 2014 to December 2016.

COLLECTION OF SAMPLES:

FECAL SAMPLE:

From each patient one freshly voided stool sample was collected in a clean, wide-mouth sterile container and examined within 2-3 hours of collection

PROCESSING OF SAMPLES:

FECAL SAMPLE:

Naked eye examination: color, consistency & presence of any parasitic forms in the stool, were noted.

MICROSCOPIC EXAMINATION:

Each sample was subjected to microscopic examination for the presence of any protozoal cyst or trophozoites, helminthic ova or larvae or any other relevant findings. Saline mount and Iodine (Lugol's) wet mount were prepared & examined under the microscope using 10x and 40x objectives & findings were noted.

FORMAL – ETHER CONCENTRATION TECHNIQUE FOR PARASITES:

Each stool sample was subjected to formal – ether concentration technique and the concentrated sediment is examined by saline wet mount, & iodine wet mount to detect the parasitic forms. Thin smear is prepared from the deposit, heat fixed and stained by modified Ziehl-Neelsen staining technique. Procedures were according to the standard methods mentioned in the appendix. [Saline wet mount, Iodine wet mount, formal ether concentration technique, modified Z-N staining].Microscopic pictures of the parasitic forms were taken on the spot, with the Binocular Microscope MOTIC B-1 series with inbuilt digital camera, available in the Department of Microbiology, fathima Institute of Medical sciences, Kadapa -Andhra Pradesh.

SALINE WET MOUNT:

Saline wet mount is made by mixing a small quantity of feces with a drop of physiological saline. It is seen under low power and high power microscopy. It is used to demonstrate helminthic eggs and larvae. It is also used to detect motile trophozoites of the intestinal protozoa.[8]

IODINE WET MOUNT:

Iodine wet mount is made by using a drop of iodine for preparation of wet mount of stool. Dobell and O'Connor's, Lugols' and D'Antonie's iodine are different types of iodine frequently used in the iodine wet mount. Iodine wet mount is mainly used for protozoal cysts. Iodine stained cysts show pale refractile nuclei, yellowish cytoplasm and brown glycogen material. The chromatoidal bodies are not clearly visible. The motility of trophozoites is inhibited in iodine wet mount.[9,10].

FORMALIN ETHER SEDIMENTATION METHOD FOR PARASITES. [9].

The advantage of this method is that formalin fixes the eggs, larvae and cysts so that they become non–infectious. The procedure consists of the following steps.

- Half teaspoon of fresh stool is taken in a 15ml screw capped test tube. It is mixed with 10% formalin and is allowed to stand for 30 minutes.
- The fecal suspension is filtered through two layers of gauze in a funnel into the centrifuge tube. Saline is added to the tube to bring the fluid level with in several millimeters of the rim of the tube. The tube is centrifuged for 10 minutes at 500g.
- The supernatant is discarded. The sediment is resuspended in saline and again centrifuged for 10 minutes at 500g.
- The sediment is resuspended in 7ml of 10%formalin. 3ml of ether or ether substitute is added to it. The tube is closed with a stopper and shaken vigorously for 30 seconds. The tube is held in such a way that the stopper is held away from the face. The stopper is removed carefully.
- The tube is centrifuged for 10 minutes at 500g. The tube is then allowed to stand in a test tube stand. Four layers are formed. The first bottom layer is the sediment which contains parasitic forms, second layer is the layer of formalin, third is the layer of fecal debris and top is the layer of ether or ether substitute.
- The layer of fecal debris is removed by an applicator stick from the site of the tube and then all the liquid is decanted leaving behind a drop of Formalin and the sediment. The sediment is mixed with formalin drop and either a wet mount is prepared for microscopic examination or a smear is made for modified Z-N staining.

MODIFIED ACID-FAST STAIN. [9,10]

The modified acid fast stain is now increasingly being used for detection and identification of Cryptosporidium, Isospora and Cyclosporaoocysts. Both hot and cold modified acid fast stains have been used with equal sensitivity. The staining solution contains:

1. Carbolfuschin: It contains basic fuschin 4gm ; phenol 8ml ; alcohol (95%) 20ml and distilled water 100ml. The carbolfuschin solution is prepared by mixing basic fuschin in the alcohol. To this mixture, water is added slowly while shaking. 8ml of phenol, which is melted in a water bath at 56 $^{\circ}$C is added to the stain with a pipette.
2. Decoloriser : It contains 5% aqueous sulphuric acid.
3. Counter stain: It contains methylene blue, 0.3gm and distilled water, 100ml. It is prepared by mixing methylene blue with distilled water

THE PROCEDURE OF HOT MODIFIED ACID-FAST STAINING METHOD CONSISTS OF FOLLOWING STEPS :

- A fecal smear is made on glass slide. It is fixed by heat by passing it over the bunsen flame repeatedly for 3 - 5 minutes.
- The heat fixed slide is flooded with carbolfuschin. The slide is heated intermittently till carbolfuschin starts steaming. Slide is allowed to stain for 7 to 9 minutes. More carbolfuschin is added to prevent drying.
- The smear is washed with distilled water and decolorized with 5% H_2SO_4 for 1 minute.
- The smear is then washed with distilled water and methylene blue is added for 1 minute as the counter stain.
- Finally the smear is washed with distilled water; water is drained off and dried and examined under the microscope using oil immersion objective.
- The acid fast oocysts of Cryptosporidium, Isospora and Cyclospora stained red with their typical morphological features. Non acid fast back ground stains blue with methylene blue.

RESULTS:

Table-1.ENTERIC PARASITES DETECTED FROM HIV POSITIVE PATIENTS AND CONTROL GROUP

Parasite species:	HIV positive [n = 75]	HIV negative [n =50]
Cryptosporidium	06 (8%)	00
Isospora	01 (1.33%)	00
Entamoebahistolytica	02 (2.67%)	03 (6%)
Giardia intestinalis	03 (4%)	02 (4%)
Ascarislumbricoides	01 (1.33%)	01 (2%)
Total	13 (17.3%)	06 (12%)

Table-2.ENTERIC PARASITES IN HIV POSITIVE PATIENTS WITH/WITHOUT DIARRHEA AND IN HIV NEGATIVE INDIVIDUALS

Parasite species:	HIV positive with diarrhea [n = 18]	HIV positive without diarrhea [n = 57]	HIV negative individuals [n = 50]
Cryptosporidium	06	00	00
Isospora	01	00	00
E.histolytica	01	01	03
G.intestinalis	01	02	02
A.lumbricoides	00	01	01
Total	09	04	06

Table- 3.RISK FACTORS, MARITAL STATUS AND OCCUPATION OF 75 HIV POSITIVE PATIENTS

Marital status	Married Unmarried	52 23
Risk factors	Promiscuity Blood transfusion I.V drug users Others[previous operation, spouse of HIV(+) person]	21 08 01 28

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Occupation	Driver	06
	Labourer	31
	Vendor	15
	Student	04
	Others	19
Circumcision in male	Circumcised	06
	Uncircumcised	49

Table- 4. AGE AND SEX WISE DISTRIBUTION OF HIV POSITIVE PATIENTS WITH INTESTINAL PARASITES

Age	Total number of patients		Parasite detected	
	Male	Female	Male	Female
15-25 yrs	19	04	02	01
26-35 yrs	16	03	02	03
36-45 yrs	11	08	02	02
46-55 yrs	08	05	01	00
>55 yrs	01	00	00	00
Total	55	20	07	06

Table-5.AGE AND SEX WISE DISTRIBUTION OF HIV NEGATIVE INDIVIDUALS WITH INTESTINAL PARASITES

Age	Total number of patients		Parasites detected	
	Male	Female	Male	Female
18-30 yrs	12	03	02	00
31-40 yrs	09	04	01	01
41-50 yrs	07	06	00	01
51-60 yrs	06	03	01	00
Total	34	16	04	02

Table-6.ASSOCIATION OF ENTERIC PARASITES AND ANTIRETROVIRAL THERAPY (ART)

Parasites	On ART	Without ART
Cryptosporidium	02	04
Isospora belli	00	01
E.histolytica	01	01
G.intestinalis	02	01
A.lumbricoides	01	00
Total	06	08

Various enteric parasites detected in HIV positive patients and HIV negative individuals, is shown in Table 1. For the purpose of statistical analysis, the enteric parasites are divided into the coccidian group (Cryptosporidium + Isospora belli) and conventional enteric parasites (E.histolytica, G.intestinalis and A.lumbricoides). There is a statistically significant relationship between the HIV status and enteric parasites. Enteric parasitic infection is more common in HIV positive individuals (Fisher's exact test, $p = 0.044$, 2 sided)

Various enteric parasites detected in HIV positive patients with /without diarrhea and in HIV negative individuals are shown in Table 2. Enteric parasitic infection is more common in HIV positive patients with diarrhea than HIV positive patient without diarrhea or HIV negative individuals. There is a statistically significant relationship between diarrhea and enteric parasites in HIV infected patients (Fisher's exact test, $p = 0.021$, 2 sided).

Details of the HIV positive patients pertaining to occupation, sexuality, marital status and other risk behaviors is recorded in Table 3. Promiscuity appears to be the highest risk factor in HIV positive group.

Association of intestinal parasitic infection and antiretroviral therapy is shown in Table-6. It is shown that, coccidian parasitic infection is more common in patients without ART. But no statistically significant relationship was found between ART status and enteric parasites (Fisher's exact test, $p = 0.286$, 2 sided).

DISCUSSION

The present study documents that infection with enteric parasites is common in the HIV positive patients having diarrhea, /in Kadapa, Andhra Pradesh. Out of 75 HIV- seropositive cases studied, 18 (24%) cases had diarrhea, (Table.2). Detection of enteric parasites from HIV positive patients having diarrhea was significantly higher (9/18, 50%) and statistically significant (Fisher's exact test, $p = 0.021$), compared to the patients without diarrhea (7.01%) and HIV negative patients (12%). A similar study conducted by Talib et al where the isolation rate of enteric parasites from HIV positive patients with diarrhea was 45.8% (11/24). [1] Prasad et al isolated 73% (19/26) of the enteric pathogens from the HIV positive patients with diarrhea. [3] In both of these studies, the presence of enteric parasites in HIV positive patients without diarrhea is significantly low, as in our study. Kumar et al isolated 36 enteric pathogens from 100 HIV positive patients having diarrhea (36%), where as in the same study isolation rate of enteric parasites from HIV positive patients without diarrhea was 14% (7/50).[2] But a similar study by Mukhopadhya et al documented that infection with enteric pathogens is common in southern Indian HIV positive patients, regardless of the presence of diarrhea and the overall isolation of enteric pathogens from stool was similar in patients with or without diarrhea.[4]

Figures from various studies demonstrate striking geographic variations in the prevalence of individual pathogens in HIV infected patients. These variations may relate both to the prevalence of pathogens with in the community and to drugs used prophylactically in patients with HIV infections. Isolation rates, in this study (Table 1) of Cryptosporidium (8%, reported rates 6 – 37%), Giardia (4%, reported rates 1 – 11%) and Isospora belli (1.33%, reported rates 0 – 3%) from HIV positive patients, were within the ranges reported from elsewhere. In some recent studies, the isolation rate of Isospora belli has shown an increasing trend – Mukhopadhya et at (18%)[4].; Kumar et al (13.7%)[2].; Prasad et al (27%)[3], compared with the previous ones – Talib et al (1.25%); Ballal et al (2.86%). The high rate of infection with Isospora belli poses a threat to HIV positive patients. The actual rate of this infection in immune compromised individuals and AIDS patients is likely to be underestimated due to asymptomatic shedding of oocysts and treatment with trimethoprim-sulphamethoxazole for other infections in AIDS patients (Pneumocystis carinii pneumonia), which may confer some protection against this parasite.

The detection rate of Cryptosporidium oocysts from HIV positive patients in our study (8%) is in accordance with the other studies (6-37%). All the stool samples positive for Cryptosporidium oocysts were diarrheal. Cryptosporidium is also the most prevalent parasite (6/13, 46.1%) in our study group of HIV positive patients. No Cryptosporidium oocysts were detected from the HIV positive patients without diarrhea or from HIV negative individuals. This finding suggests that intestinal infestation with Cryptosporidium spp is associated with diarrhea in HIV positive patients. A similar study conducted by Mukhopadhya et al and Kumar et al where the isolation rate of Cryptosporidium oocysts from HIV positive patients without diarrhea was 6% and 8% respectively. But Talib et al also documented that all the Cryptosporidium positive stool samples were diarrheal.

shows the presence of risk factors, marital status and occupation of 75 HIV positive patients. In our study group maximum patients were laborer (41%). Promiscuity appeared to be the highest risk factor among the study group. Among the 55 male patients only 6 patients had circumcision while 49 patients were uncircumcised. As the socioeconomic status of our study group was very poor, so we were unable to get information on the CD_4+ count of the patients. For four patients, the CD_4 + T lymphocyte count done elsewhere were recorded and all of them were above 200/μl. So the relation between CD4+ count and presence of intestinal parasites could not be established. The earlier study showed that, intestinal opportunistic protozoal infection and diarrhea is more common in patients with low CD4+ count. [11,12,13]

Another important finding in our study was that all the microscopy positive stool samples for Cryptosporidium oocysts appeared dark green to olive green in colour and watery to semiformed in consistency. The same findings were observed by Nagamani et al in her study on "Molecular Characterization of Cryptosporidium between 2003 and 2006".[14] It was also observed in a few patients that as these samples became negative on microscopy, the character of stool changed from watery green to formed yellow colour.[15]

We were unable to identify any mixed enteric infection in our study though there are reports about mixed or coinfection in few other studies. Shah et al reported a case of AIDS from Gujarat with

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coinfection with Cryptosporidium, Isospora and S. stercoralis.[16] Prasad et al identified two mixed infections, Isospora belli with S. stercoralis in one and the other is Cryptosporidium with E. histolytica.[3][17] Kumar et al reported 3 cases of mixed infection with enteric pathogens in HIV positive patients which includes 2 cases of mixed infection with Cryptosporidium and Isospora belli and one case of Cryptosporidium with Ancylostomaduodenale [2] [18].The mixed infections depend on multiple factors like severe immunosuppression, poor sanitary condition and geographical variations.

SUMMARY AND CONCLUSION

1. The study period of one year and eight months revealed that it is difficult to locate / identify a HIV positive person in a place like Kadapa on account of confidentiality / lack of awareness among the population.
2. Having identified the HIV positive cases, collection of stool samples from the patients also needed counseling and intervention by the respective clinicians especially when patients were not having any symptoms.
3. Follow up of the HIV positive cases is difficult as they lose contact with the clinicians and not traceable after being discharged from the hospital.
4. As there was difficulty in identifying the HIV positive cases, having located a case we collected stool samples from the patients.
5. Detection of intestinal parasites is 17.3% in HIV positive patients and 12% in HIV negative individuals.
6. In HIV positive cases with diarrhea, detection of intestinal parasites is 50% where as in HIV positive cases without diarrhea it is only 7.01%.
7. Coccidian parasites Cryptosporidium and Isospora are detected only in HIV positive cases with diarrhea. They are not detected in HIV positive cases without diarrhea or HIV negative individuals.

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