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INCIDENCE OF ENTERIC PATHOGENS WITH SPECIAL REFERENCE TO CRYPTOSPORIDIOSIS IN HIV INFECTED PATIENTS WITH DIARRHOEA

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ABSTRACT: BACKGROUND: Opportunistic infections (OIs) have been recognized as common complications of HIV infection since the beginning of the acquired immunodeficiency syndrome (AIDS) pandemic. Infection of gastro intestinal tract leading to chronic diarrhoea and malnutrition is quiet common in patients with AIDS. **AIMS:** This study was conducted to identify the demography, the common etiological agents and various emerging pathogens causing diarrhoea in HIV infected individuals with special reference to cryptosporidiosis. **Methods and materials:** Study was conducted on 100 HIV positive cases with diarrhoea and 100 HIV negative patients with diarrhoea as controls. Detailed demographic data and clinical history was taken. The identification of pathogens was done on the basis of direct microscopy and different staining techniques. **RESULTS:** Enteric infections were detected in 81% of the stool samples of HIV-positive diarrhoea patients and in 30% of HIV-negative patients with diarrhoea. Cryptosporidium was isolated from 56 out of 100 HIV positive cases with diarrhoea while the isolation rate is only 16% in HIV negative controls. Most among HIV positive cases with diarrhoea were illiterates and hail from rural areas. **CONCLUSION:** Infection of Cryptosporidium was significantly higher among HIV-positive subjects with diarrhoea. Screening for these infections is therefore essential for early diagnosis and treatment.

KEYWORDS: Human immunodeficiency virus, Cryptosporidiosis, Diarrhoea.

INTRODUCTION: Human immunodeficiency virus (HIV), since its isolation in 1983 has turned out to be the world's worst biological holocaust. HIV infection has globally claimed over 20 million lives and 40 million people carry the infection.¹ In India the overall HIV prevalence is 0.9%.²

Opportunistic infections (OIs) have been recognized as common complications of HIV infection since the beginning of the acquired immunodeficiency syndrome (AIDS) pandemic.³

Infection of gastro intestinal tract leading to chronic diarrhoea and malnutrition is quiet common in patients with AIDS. It has been estimated that 30-50% of patients with AIDS in USA and about 90% in Africa and Haiti suffer from chronic diarrhoea⁴. The isolation rates of intestinal parasites in patients with AIDS and chronic diarrhoea vary from 40 to 83% and the causative agents also differ from region to region depending on incidence of intercurrent infections and hygiene.^{5,6}

The etiological spectrum of enteric pathogens causing diarrhoea includes bacteria, parasites, fungi, and viruses.⁷ Opportunistic parasites like Cryptosporidium parvum, Cyclospora cayetanensis, Isospora belli, and Microsporidium sp are documented in patients with AIDS.⁸ Non-opportunistic parasites such as Entamoeba histolytica, Giardia intestinalis, Trichuris trichiura, Ascaris lumbricoides, Strongyloides stercoralis, and Ancylostoma duodenale are frequently encountered in developing countries but are not currently considered opportunistic in AIDS patients.⁹

Diarrhoea is considered significant when the stool output is greater than 500gms per day. Diarrhoea may be mild and intermittent, or severe and disabling; spontaneous exacerbations and

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remissions are common. Chronic diarrhoea significantly reduces the quality of life in patients with HIV infection,¹⁰ and is an independent predictor of mortality in AIDS.^{11,12} Large volume diarrhoea, frequently pale and bulky, is generally associated with small bowel disease such as seen with cryptosporidiosis. Frequent small volume stools with tenesmus, hematochezia or rectal discharge may suggest herpes simplex proctitis, invasive viral or bacterial colitis. Diarrhoea may also be the result of neoplasm, HIV enteropathy or drug toxicity.

Cryptosporidium parvum, a unicellular, spore forming coccidian protozoan causes chronic diarrhoea especially in patients with CD4 T lymphocyte count of less than 200/mm³. The disease is easily spread by contaminated food or water or by direct contact with an infected person or animal. The living conditions of the people have a great influence on the transmission of the parasite in the community. Infection results in cholera like watery diarrhoea or mucoid diarrhoea in immunocompromised patients. The duration of diarrhoea in immunocompromised individual is 20 weeks with variability between 1 and 48 weeks and is accompanied by low grade fever, nausea, vomitings and abdominal cramps.

This study was conducted to identify the demography, the common etiological agents and various emerging pathogens causing diarrhoea in HIV infected individuals with special reference to cryptosporidiosis.

MATERIALS AND METHODS:

Study Design: Study was conducted from January 2011 to December 2011, in the Department of Dermatology, Venereology and Leprosy, Government General Hospital, Vijayawada, Andhra Pradesh, India. The study group included:

- a. 100 HIV seropositive patients with diarrhoea- cases.
- b. 100 HIV seronegative patients with diarrhoea- controls.

A detailed history of the patient including demography, complete clinical history, sexual history, past history of sexually transmitted diseases, blood transfusions, hospitalisation, I.V drug abuse and tattooing was taken and a thorough clinical examination was performed.

Inclusion Criteria: Diarrhoea was defined as two or more liquid or three or more soft stools per day. The duration and frequency of diarrhoea were noted to classify it as acute if it is lasted for less than 1 month and chronic if it is lasted for more than 1 month.

Exclusion Criteria: Persons who received antiparasitic treatment for diarrhoea in the past 14 days were excluded.

Stool Examination: Stool specimens were collected in labeled, leak proof, clean sterile plastic containers and processed within 4 hours in the clinical parasitology laboratory. The consistency of stool samples was noted. Wet mount of stool in normal saline (0.85%) was prepared and examined for the presence of motile intestinal parasites and trophozoites under light microscope. Lugol's iodine staining was used to detect cysts of intestinal parasites.

Both wet mount and iodine smear were prepared from unconcentrated as well as formal-ethyl acetate concentrated stool samples. Air dried thin smears were stained by modified acid fast

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staining technique for identifying coccidian parasites (*Cryptosporidium*, *Cyclospora*, *Isospora*). Gram's staining was done. All stool samples were routinely cultured on MacConkey, Deoxy chocolate citrate agar, blood agar and selenite F broth. All the inoculated media were incubated for 16 to 18 hours at 37°C after which detailed examination of the morphology of colonies was done. The specific pathogens were confirmed by slide agglutination test with standard high titre sera.

RESULTS: Of the 100 HIV positive cases with diarrhoea, 50 were males and 50 were females. Most male patients were in age group of 26-35 years i.e 23(46%) followed by 15-25 years i.e 15 (30%), while most females were in age group of 15-25 years i.e 29 (58%) followed by 26-35 years i.e 11 (22%) [Table 1].

68% of males and 84% of females among HIV positive cases were illiterates. The percentages of male and female cases from rural areas were 76% and 84% respectively. Out of 100 HIV positive cases 90% of males and 76% of females were married, 10% of males were unmarried, 8% of females were separated and 16% of females were widows. Of the total 100 HIV positive cases 9% had premarital exposure, 37% had extramarital exposure, 13% had both, 36% (All females) gave history of spouse having extramarital exposure. Only 5% denied extramarital exposure whereas all HIV negative individuals with diarrhoea denied pre or extramarital exposure. Probable mode of transmission of HIV among the cases was heterosexual exposure in 92% (42% males, 50% females), homosexual transmission in 6% of males, blood transfusion in 6% of males and through major surgery in 4% of males. 30(60%) of males, 21(42%) of females had past h/o STD (Table 2) while 32 (64%) of males, 33(66 %) of females had STD at the time of study (Table 3). Most of them are Viral STD.

Out of 100 HIV positive cases, 40% had acute diarrhoea and 60% had chronic diarrhoea as compared to 72% with acute and 28% with chronic diarrhoea in HIV negative controls. Among HIV positive cases 80% had watery diarrhoea with or without blood and 20% had mucoid diarrhoea with or without blood. 80% of HIV positive cases with diarrhoea also had associated fever, abdominal pain and vomiting, 18% had only abdominal pain and vomiting and 2% had no associated symptoms.

Cryptosporidium was isolated from 56 out of 100 HIV positive cases with diarrhoea while the isolation rate is only 16% in HIV negative controls. *Entamoeba histolytica* was isolated in 15%, *Candida* species in 3%, *Ascariasis lumbricoides* in 3%, *Giardia intestinalis* in 2%, *Klebsiella* species *Salmonella typhimurium* in 1% each. Stool samples were negative for *Isospora belli*, *Strongyloides stercoralis*, *Trichuris trichiura*, *Microsporidium*, *Cyclospora*. In HIV negative patients with diarrhoea *Entamoeba histolytica* was isolated in 10%, *Ascariasis lumbricoides* in 2%. *Giardia intestinalis* and *Salmonella typhimurium* in 1% each [table 4]. Enteric infections were detected in 81% of the stool samples of HIV-positive diarrhoea patients and in 30% of HIV-negative patients with diarrhoea.

DISCUSSION: This study reports the incidence of enteric pathogens in HIV positive cases with diarrhoea with special reference to *Cryptosporidiosis* in addition to the clinico-epidemiological characterization of the cases. Since diarrhoea is an important manifestation of the intestinal infection in HIV-infected patients, we compared the incidence of various etiological agents with that of HIV-negative patients with diarrhoea (81% stool samples are positive in cases compared to 30% of controls). This association is in agreement with other studies which have shown that pathogens are important etiological agents of diarrhoea in HIV-positive patients.^{13,14,15}

Majority of HIV positive diarrhoea cases are in the sexually active age group i.e between 15-35 years (78%). *Cryptosporidiosis* is common in HIV positive young individuals when compared to

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HIV negative controls where isolation rate is common in individuals above 45 years. Illiterates (76%) and individuals with primary education (26%) formed major group in our series which reflects lack of awareness of safer sex practices. In both cases (80%) and controls (61%) majority of patients were from rural areas. Factors like overcrowding, inadequate nutrition and poverty were predominant in this group. Harish Khubnan, K. Sivarajan et al study reported 1.36% of cryptosporidium positive cases in humans and 10.89% of cryptosporidiosis in cattle. He assumed that in rural areas most likely source of cryptosporidium for human infection is cattle.

In 100 cases, 83% were married, 5% unmarried, 4% separated and 8% were widows. This correlated with Ramesh Chandra Gharani, Bhagat Ram Garg et al study i.e., married 67.8%, unmarried 25.4% widows 1.7%, widowers 5.1%. Among HIV positive cases, 9% had premarital exposure, 37% had extramarital exposure, 13% had both and 36% (all females) gave history of spouse having extramarital exposure. Only 5% denied extramarital exposure whereas all HIV negative individuals with diarrhoea denied pre or extramarital exposure. According to King K Holmes, premarital exposure increases the number of life time sex partners and contribute to epidemic spread of HIV/STD. The present study shows increased risk behaviour and promiscuity in males compared to females.

In the present study probable mode of transmission is heterosexual exposure in 92% (42% males, 50% females), homosexual transmission in 6% of males, blood transfusion in 6% of males and through major surgery in 4% of males. None had history of intravenous drug injections. This is in accordance with King K Holmes et al study which states that overall predominant route of transmission is heterosexual exposure. Several prospective studies have linked the presence of genital ulcer disease to HIV seroconversion and one study showed significant association between genital ulcer diseases in HIV infected men and the transmission of HIV to their female partners.

Chronic diarrhoea predominated (60%) among HIV positive cases in comparison to HIV negative controls (28%). In the present study, enteric pathogens were detected in 81% of HIV-infected patients with diarrhoea and 30% of HIV-negative patients with diarrhoea. This study corroborates well with other studies which reported a prevalence of intestinal parasites, ranging from 30% to 60% in the HIV-positive diarrhoea patients. Cryptosporidium was isolated in 56% of cases and in 16% of controls. Fisseha B, Petro S.B et al. of Ethiopia in 1998 reported prevalence of cryptosporidiosis in HIV positive diarrhoea cases as 29.9%. Brandonisio, Maggi P, Panoro MA, et al in Italy reported prevalence of cryptosporidiosis as 33.3% in HIV positive cases.

The prevalence of cryptosporidiosis in Africa and Haiti is 50% which almost correlated with our study. Lack of education, poverty and unhygienic conditions prevailing in the developing countries may be responsible for the high prevalence rates of cryptosporidiosis in these countries. K.N. Prasad, V.L. Nag, T.N Dhole et al study reported prevalence of 11%. Anand L, Dhanachand C, Brajachand N of Manipur reported a high prevalence of 94.4% in asymptomatic HIV positive injection drug users.

Isospora belli was not isolated in either cases or controls in the present study.

The prevalence of *Isospora belli* according to K.N Prasad et al study from Lucknow was 31%, D.N. Lanjewar et al from Bombay was 13%, Fisseha et al study from Ethiopia was 1.4%. Widespread use of trimethoprim sulphamethoxazole as prophylactic agent in HIV individuals which is also effective against *Isospora belli* may be responsible for the above observation in the present study.

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In K.N. Prasad, V.L Nag, T.N Dhole et al study from India no cyclospora and microsporidium was isolated. This correlates with the present study. In the same study K.N Prasad et al reported *Entamoeba histolytica* in 7.7% which is lower than that in the present study (15%), and *Giardia* (3.8%) which is higher than that in the present study (2%).

D.N. Lanjewar, Rodrigues, D.G Saple et al study from Bombay reported prevalence of *Strongyloides* as 4% which is higher than that in our study.

CONCLUSION: The high prevalence of intestinal parasitic infections in HIV-positive patients warrants urgent need of intervention so as to avoid its consequences. Infection of *Cryptosporidium* was significantly higher among HIV-positive subjects with diarrhoea. Screening for these infections is therefore essential for early diagnosis and treatment.

REFERENCES:

1. UNAIDS. Report on the Global AIDS Epidemic. Geneva: UNAIDS; 2007.
2. Guidelines on HIV testing manual. National AIDS control Organization. Ministry of health and family Welfare. Govt. of India. Available from: <http://nacoonline.org/AboutNACO/Policy-Guidelines/Policies> [Last accessed on 2007 Mar].
3. Janoff EN, Smith PD. Perspectives on gastrointestinal infections in AIDS. *Gastroenterol Clin North Am* 1988; 17: 451-63.
4. Framm SR, Soave R. Agents of diarrhoea. *Med Clin North Am* 1997; 81: 427-47.
5. Sharpstone D, Gazzard B. Gastrointestinal manifestations of HIV infection. *Lancet* 1996; 348: 379-83.
6. ICDDR, B. Centre for Health and Population Research- Identification of Enteric pathogens in HIV positive patients with diarrhoea in Northern India- K. N. Prasad, V. L. Nag, T. N Dhole and A. Ayyagari- Department of Microbiology, Sanjay Gandhi Postgraduate Institute of Medixcal Sciences, Lucknow.
7. Mitra AK, Hernandez CD, Hernandez CA, Siddiq Z. Management of diarrhoea in HIV-infected patients. *Int J STD AIDS* 2001; 12: 630-9.
8. Goodgame RW. Understanding intestinal spore-forming protozoa: *Cryptosporidia*, *microsporidia*, *Isospora* and *Cyclospora*. *Ann Intern Med* 1996; 124: 429-41.
9. Lucas SB. Missing infections in AIDS. *Trans R Soc Trop Med Hyg* 1990; 8 Suppl 1:34-8.
10. Lubeck DP, Bennett CL, Mazonsen PD, Fifer SK, Fries JF. Quality of life and health service use among HIV infected patients with chronic diarrhoea. *J Acquir Immune Defic Syndr* 1993; 6: 478-84.
11. Justice AC, Feinstein AR, Wells CK. A new prognostic staging system for the acquired immunodeficiency syndrome. *N Engl J Med* 1989; 320: 1388-93.
12. Lindan CP, Allen S, Serufulira A, Lifson AR, Van de Perre P, Chen Rundle A et al. Predictors of mortality among HIV infected women in Kigali, Rwanda. *Ann Intern Med* 1992; 116: 320-8.
13. Lew FA, Poles MA, Dieterich DT. Diarrheal disease associated with HIV infection. *Gastroenterol Clin North Am* 1997; 26: 259-90.
14. Tuli L, Gulati AK, Sundar S, Mohapatra TM. Correlation between CD4 counts of HIV patients and enteric protozoan in different seasons - an experience of a tertiary care hospital in Varanasi (India). *BMC Gastroenterol* 2008; 8: 36.

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15. Mukhopadhyaya A, Ramakrishnan BS, Kang G, Pulimood AB, Mathan MM, Zachariah A, et al. Enteric pathogens in southern Indian HIV-infected patients with and without diarrhoea. Indian J Med Res 1999; 109: 85-9.

Age group	HIV positive cases			HIV negative controls		
	Males	Females	Total	Males	Females	Total
15-25 years	15(30%)	29(58%)	44%	04(08%)	15(30%)	19%
26-35 years	23(46%)	11(22%)	34%	21(42%)	03(06%)	24%
36-45 years	08(16%)	03(06%)	11%	17(34%)	09(18%)	26%
Above 45yrs	04(08%)	07(14%)	11%	08(16%)	23(46%)	31%
Total	50	50	100	50	50	100

Table 1: Age and sex distribution of cases and controls with diarrhoea

Sl. No.	Past history of s.td	Males	Females	Total	Percentage
1	Non-herpetic ulcer	02	01	03	03%
2	Urethral Discharge	08	07	15	15%
3	Inguinal bubo	01	00	01	01%
4	Condyloma acuminata / genital warts	05	02	07	07%
5	Herpes genitalis	08	06	14	14%
6	Molluscum contagiosum	06	05	11	11%
Total no. of cases had past H/O of STD		30(60%)	21(42)	51	51%
Total no. of cases had no past history of STD		20	29	49	49%
	Total	50	50	100	100%

Table 2: Past history of STDs in HIV positive cases with diarrhoea

30(60%) of males, 21(42%) of females had past history STD

Sl. No.	Present STD in HIV patients	Males	Females	Total	Percentage
1	Candidal balanoposthitis/ candidal vulvovaginitis	2	5	7	7%
2	Condylomata acuminata / genital warts	12	10	22	22%
3	Herpes genitalis	12	13	25	25%
4	Molluscum contagiosum	6	5	11	11%
Total No. of cases having present H/O STD		32(64%)	33(66%)	65	65%
Total No. of cases with Nil STD at present		18	17	35	35%
	Total	50	50	100	100%

Table 3: Present history of STDs in HIV positive cases with diarrhoea

32(64%) of males, 33(66%) of females having present history of STD. Most of them are viral STD.

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Enteric pathogens	HIV positive cases	%	HIV negative cases	Percentage
1. Cryptosporidium	56	56%	16	16%
2. Entamoeba histolytica	15	15%	10	10%
3. Candida species	03	03%	0	0
4. Ascariasis lumbricoides	03	03%	02	02%
5. Giardia intestinalis	02	02%	01	01%
6. Klebsiella species	01	01%	0	0
7. Salmonella typhimurium	01	01%	01	01%
8. Isospora belli	0	0	0	0
9. Strongyloides stercoralis	0	0	0	0
10. Trichuris trichiura	0	0	0	0
11. Microsporidium	0	0	0	0
12. Cyclospora	0	0	0	0
Total	81	81%	30	30%

Table 4: Incidence of various pathogens isolated from HIV positive cases and HIV negative controls

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