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Prevalence of Opportunistic Infection of Intestinal Parasitic and Bacteriae n HIV-1 /AIDS Positive Individuals in Kadapa.

Obulesu G[1]*, Hunumanthappa[2], and Prabakar Reddy[3].

[1]PhD Scholor,Barath Univarsity, Chennai
[2]Professor in Microbiology, JJM Medical College, Davangere, Karnataka, India
[3]Associate Professor in Biochemistry Sri Lakshmi Narayana Inistitute Of Medical sciences, Pondycherry ,India

ABSTRACT

Intestinal parasities and bacterial predominant in HIV positive patient, CD4 cell count variation. Study was conducted during 2014 YEAR HIV positive cases ,microscopically , bacteriological culture, and CD4 cell count. 74% of HIV seropositive individuals from study group belonged to low socio economic status and 26% belonged to middle socioeconomic status.occupational status among 100 HIV seropositive individuals studied, 45.58% (31/68) of males and 53.12% (17/32) of females were labourers. 23.52% (16/68) of males were drivers. 5.88% (4/68) of males were businessmen. 11.76% (8/68) of males were farmers. 8.82% (6/68) of males and 3.12% (1/32) of females were employees.4.41% (3/68) of males were unemployed. 43.75% (14/32) of females were housewives. Isosporaoocyst was the predominant parasite detected in stool samples 28 (40%), followed by cryptosporidium 15(21%) Strongyloides larvae 12 (17%)and Ascaris ova 11 (17%) each, E.histolytica cyst & Giardia trophozoites

Keywords: HIV/AIDS, , opportunistic parasite

Corresponding author:

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INTRODUCTION

HIV Virus caused by AIDS. It is a serious disorder of the immune system, where the body's normal defences against infection break down leaving the host vulnerable to life threatening infections and unusual malignancies.

The virus has spread virtually all over the world. The HIV epidemic spread differs both in the mode of infection and its clinical manifestations between the developed and developing countries. According to the latest statistics in the world epidemic of HIV/AIDS by UNAIDS/WHO, The cardinal features of HIV infection is the depletion of T – helper /inducers lymphocytes, is due to the tropism of HIV for the population of lymphocytes which express the CD4 phenotypic marker on their surface[1].

Clinical manifestations in HIV infections are primarily not due to viral cytopathology but are secondary to failure of immune response. Several infective organisms responsible for opportunistic infections differ in characteristics from that of conventional communicable disease and are mainly low or non virulent, Specific antimicrobial prophylaxis, by itself or in combination with antiretroviral therapy, can reduce the substantial morbidity and mortality caused by opportunistic infections in patients with HIVEarly diagnosis of opportunistic infections and prompt treatment definitely contributes to increased life expectancy among infected patients delaying the progression of HIV to infected AIDS[2].

MATERIALS AND METHODS

A total number of 300 stools, samples were collected from 300 HIV seropositive patients belonging to stage III and IV as screened by ICTC&ART centre, KADAPA. They were also advised to undergo CD_4 counts in the Department of **Microbiology**, **Fathima institute Of Medical Sciences, Kadapa**

Methodology

1. **Collection of samples**
2. **Direct Microscopy**

   a. Modified Ziehl-Neelsen staining for stool.
   b. Saline mount & Iodine mounts for stool.

3. **Tests for Identification of isolates**
   MEDIA FOR ISOLATION

CD4&CD8 COUNTS

Material collection: The sample collected for enumeration of CD4 & CD8 counts was whole blood drawn with sterile disposable syringe with aseptic precautions.3ml blood was withdrawn by venipuncture in a k3 EDTA [liquid] vacutainer tube.

Storage: The sample was run with BECTON DICKINSON FACS within 48 hours of blood collection

System for counting CD4 && CD8 cells:

Lymphocyte sub setting FACS count system:

FACS count system is a dedicated compact system for automatically counting CD4+, CD8+ and CD3+ T-lymphocytes, which are used to monitor the immune status of HIV, infected patients. The compact, self-constricted system, incorporating reagents and controls, eliminates the need for hematology results to obtain absolute lymphocyte count values and simplifies the sample preparation process[5]. The FACS count system uses whole blood eliminating lyses and wash steps. A unique software algorithm identifies the lymphocyte population of interest automatically. The system is easy to use, cost effective and reliable in the clinical laboratory. The system is designed to utilize the power and advantages of flow cytometry. In just few steps

complete T-lymphocytes panel – absolute counts of CD4+, CD8+and CD3+T- lymphocytes, as well as the helper/suppressor ratio (CD4+/CD8+) can be obtained[6]

The instrument is connected to standard electrical outlet and requires no external computer or user adjustment to hardware or calibration.

FACS count system components:

1. FACS count instrument.
2. FACS count spare parts kit.
3. FACS count information kit.
4. FACS count user's guide.
5. FACS count system quick reference guide.
6. FACS count software.
7. FACS count work station: Compact work station that holds samples and reagents.
8. FACS count coring station: Device used for opening the inner membrane of theCD4& CD8 tubes.
9. FACS count pipette: Pre programmed electronic pipetter for reverse pipetting.
10. Vortex mixer use for mixing the reagents by creating swirling motion.
11. System fluid.
12. Pipette tips.
13. Reagents:

These substances are used for preparing whole blood samples. FACS count reagents (CD4/CD3 and CD8/CD3) are contained in two paired reagent tubes. The fluorochrome conjugated monoclonal antibody reagent is a 0.4ml buffered solution with stabilizer and 0.1% sodium azide. Helper / inducer T-lymphocytes clone is identified by yellow-orange-labelled CD4, clone SK3 and suppressor/cytotoxic T-lymphocytes clone is identified by yellow-orange-labelled CD8, clone SK1 and T- lymphocyte clone identified by red-labelled CD3, clone SK7[7].

Storage: Reagents are stored at 2 – 8^0c temperature.

Preparing patients samples:

1. The reagent pair tube was labelled with patient accession number.
2. The reagent pair was then vortexed upside down for 5seconds then upright for 5seconds.
3. Then the reagent tubes were opened with coring station.
4. The patient's whole blood was mixed by inverting vacutainer 5times.
5. By using FACS count electronic pipette 50ul of patient whole blood was pipette into each tube.
6. The reagent pair tubes were capped and vortexed upright for 5seconds.
7. The tubes were incubated for 60 min. at room temperature in dark.
8. After incubation the tubes were uncapped and 50ul of fixative solution pipetted into each tube.
9. The tubes were recapped and vortexed for 5seconds.
10. The prepared samples were run on FACS count instrument.

Entering patient and reagent information on FACS count system:

1. FACS count screen for running patient sample 'SAMPLE' was pressed.
2. After verifying reagent lot code and bead counts 'CONFIRM' was pressed on FACS count screen.
3. Then patient accession number was entered.

Running patient's samples:

1. The reagent was vortexed for 5seconds.
2. The CD4 tube was uncapped and placed in sample holder so that the CD4+ tube was in run position.
3. The sample was taken up by FACS count on pressing RUN.

4. When sample holder came down CD4 tube was recapped and CD8 tube was uncapped and placed in sample holder so that CD8 tube was in run position.
5. The sample was taken by FACS count on pressing 'RUN'.
6. When the sample holder came down, the reagent pair tube was removed and discarded into appropriate biohazard container[8].

Reading of the results:

Sample results printout:

The patient results were displayed on the screen and printed out automatically. The sample printout contains the following information.

1. Reagent Information – reagent lot code and reference bead counts entered for the sample run.
2. Date and time when sample was run.
3. Control Information-control run results, date of control run, reagent lot code entered for the control run, control lot code.
4. Patient accession number.
5. Patient results.

e.g.: Patient results

RESULTS

Out of 300 HIV seropositive individuals studied, 68% were male and 32% were females. 90.62% (87/96) of the females were in 21-40 years age group. 86.35% (131/204) of males were in 21-40 years age group. 86% (258/300) of individuals were between the age group 21- 40 years. 3.03 %(6/204) of males were in age group > 50 years & no females were present in this group

Out of three hundred cases of HIV seropositive individuals studied, 189 individuals were from rural areas (63%) and 111 individuals were from urban areas (37%). 74% of HIV seropositive individuals from study group belonged to low socio economic status and 26% belonged to middle socioeconomic status.

Occupational status among 300 HIV seropositive individuals studied, 45.58% (93/204) of males and 53.12% (51--96) of females were labourers. 23.52% (48/204) of males were drivers. 5.88% (12/204) of males were businessmen. 11.76% (24/204) of males were farmers. 8.82% (18/204) of males and 3.12% (3/96) of females were employees.4.41% (9/204) of males were unemployed. 43.75% (42/96) of females were housewives.

Table 1 Mode of transmission (n=100).

Sl. No	Mode of transmission	Number of cases	%
1.	Heterosexual	300	100
2.	Homosexual	-	-
3.	IVDU	-	-
4.	Blood transfusion	-	-

Shows the mode of transmission among the 100 HIV seropositive individuals. 100% (100/100) had heterosexual route of transmission, there were no homosexuals or intravenous drug users and no history of blood transmits

Table: 2 Sex wise pattern of CD4counts in HIV positive patients (n=300).

CD4	Male	%	Female	%	Total
>500/mm^3	15	7.35	12	12.5	27
200 –500/mm^3	42	20.58	18	18.75	60
50 – 200/mm^3	90	44.11	36	37.5	126
<50/mm^3	76	27.94	30	31.25	106

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.**Table: 3**: **Total number of samples collected -culture positivity (n=).**

Sl. No	Samples	Total Number	Culture / smear Positive	%
1	Stool	300	234	78

Total 300 Samples were collected. Out of 300 stool samples 234 (78%) were culture/smears positive.

Stool samples:

Table: 04: Parasites detected in stool samples (n=78)

Sl. No.	parasites	No. of cases	%
1.	*Isospora belli oocyst*	84	40
2	*cryptosporidium*	45	21
3	*Strongyloidesstercoralis larva*	36	17
4.	*Ascaris ova*	33	16
5.	*E. histolytica cyst*	18	08
6	*Giardia trophozoites*	18	08

Isosporaoocyst was the predominant parasite detected in stool samples 84(40%), followed by *cryptosporidium* 45(21%)*Strongyloides* larvae 36 (17%)and *Ascaris* ova 33 (17%) each, *E.histolytica cyst & Giardia trophozoites*18(08%) each.

Table: 05: **Bacterial isolates from stool samples (n=252).**

Sl. No.	Parasites	No. of cases	%
1.	*E .coli*	120	39
2.	*Shigellaflexneri*	63	25
3.	*Pseudomonasaeruginosa*	63	25
4.	*Enterococcusfaecalis*	36	14

Table: 06: **Mixed bacteria and parasites detected from stool samples (n=39).**

Sl. No.	Parasites	No. of cases	%
1.	*Isospora+ Strongyloides*	15	38
2.	*G.lamblia +Pseu.aeruginosa*	15	38
3.	*E. histolytica+ E. coli*	09	23

Isospora belli and *Strongyloides larvae* were detected from 15case (38%) followed by *Giardia lamblia& Pseudomonas aeruginosa* 15(38%) and *E. coli & E. histolytica* from 09(23%) each

Table: 07: **Antibiotic sensitivity pattern of Gram negative bacterial isolates (n=41).**

Organisms tested	No of strains tested	No. of Strains sensitive													
		A		Ak		G		Cf		Cp		Cu		T	
		No	%	No	%	No	%	No	%	No	%	No	%	No	%
Pseudomonas aeruginosa	36	-	-	27	75	21	58.3	06	22.2	03	11.1	27	75	27	75
E .coli	45	R	-	45	100	30	66	30	66	30	66.6	45	100	15	100
Shigellaflexneri	21	9	42	21	100	21	100	21	100	9	42	21	100	21	100

(R=Resistant; – = not tested)

Pseudomonas aeruginosa were 77.77% (27/36) sensitive to Amikacin, 58.33% (21/36) to *Gentamycin,* 25% (9/36) sensitive to Ciprofloxacin, 11.11% (3/21) sensitive to Cephalexin and 77.77% (27/36) sensitive to Cefuroxime and Tetracycline. E. coli totally resistant to Ampicillin, 100% sensitive to Amikacin ,Cefuroxime, Tetracycline and 66% (30/45) sensitive to Gentamycin, Ciprofloxacin and Cephalexin .Shigellaflexneri were 42% (9/21) sensitive to Ampicillin & Cephalexin and 100% (6/6) sensitive to Amikacin, Gentamycin Ciprofloxacin, Cefuroxime and Tetracycline.

Table: 08 Antibiotic sensitivity pattern of Gram positive bacterial isolates (n=135).

Organisms	No of Strains tested	No. of strains sensitive													
		A		Cfs		E		Cx		Ak		G		CP	
		No	%	No	%	No	%	No	%	No	%	No	%	No	%
Enterococci spp	12	R	-	4	100	R	-	R	-	4	100	4	100	4	100

(R=Resistant)

Enterococci spp was totally resistant to Ampicillin, *Erythromycin, Cloxacillin* and 100% sensitive to Cefperazone+ Sulbactam, Amikacin, Gentamycin and Cephalexin.

Table: 09 Isolates obtained from study & control group.

S. No	Study Group	Control Group
III.	**Parasites detected**:-	
1.	*Isospora belli*	-
2.	*Strongyloides larvae*	-
3.	*E.histolytica*	-
4.	*Giardialamblia*	Giardia
5.	*Ascarislumbricoides*	Ascaris

Parasites detected in control group are Giardia and Ascaris. Opportunistic parasites were not detected in control group

DISCUSSION

Samples were collected 300 HIV seropositive individuals.. Control group of HIV seronegative patients with similar symptomatology were also studied for various pathogens.

Sex vise :shows 84% of individuals were between 21-40 yrs age group, which is sexually active age group and male: female ratio was 2:1.This observation matches with the findings of Kumarasamy N et al (1995) hennai who reported M: F as 2:1. Locality 63% of seropositive individuals were from rural area and 37% were from urban, which was consistent with the findings of Aruna Aggarwal et al (2005) Punjab who reported 77% from rural area and 33% from urban area.

Shows 74% of individuals belong to low socioeconomic status. This observation coincides with findings of Singh A et al (2002) Manipal shows 54% of individuals were illiterates. 23% of individuals had primary education. Many of them were labourers and drivers (48% and 16% respectively); migrating from place to place and staying away from home for long time made them indulge in high risk sexual behavior.

Shows heterosexual route as the commonest mode of transmission (100%) in the present study, this coincides with finding of George J. et al (1996) from Pondicherry reported 96.7% and Kumarasamy N et al (1995) from Chennai who reported 94%.

Table 2 shows CD4 counts of 100 seropositive individuals.9% of individuals had CD4count >500/mm^3, 20% had CD4 200-500/mm^3, 42% had CD4 count 50-200/mm^3 and 29% had CD4 count <50/mm^3.

Prevalence of diarrheal infections was common in the present study. 33 individuals gave history of chronic diarrhea, out of them 15 had CD4 count 50-200/ mm^3& 10 had CD4 < 50/mm^3.30 individuals gave history chronic fever 18 of them had CD_4 count <50/ mm^3

Table 2 shows the total number of samples collected from patients and the percentage of culture positivity., 78% (234/300) from stool samples,

In the present study Isospora 40 %(84/234) was the predominant parasite detected from stool samples. This coincides with Chowdhary et al (2002) Mumbai reported 32%. Reports from other authors included- Mohandas et al (2002) Chandigarh - 2.5%, Cameraman S et al (1999) South Italy reported 54%.

Strongyloidesstercoralis larvae 17% (36/234) were detected in the present study and other authors reported 2.5% by Cimerman S et al (1999) Brazil, Chowdhary et al (2002) Mumbai reported 7% and Mohammed Reza Zali et al (2004) Iran reported 0.9%.

Ascaris ova were observed in 16% (33/234) in the present study. Other authors reported 22.2% by Brandonisio O et al (1999) S. Italy, Chowdhary et al (2002) Mumbai reported 3%, and Cimerman S et al (1999) Brazil reported 2.5%.

Giardia lamblia cysts were detected in 08% (18/234) in the present study; other authors reported 16% by Cimerman S et al (1999) Brazil, 8.3% by Mohammed Reza Zali et al (2004) Iran, 5% by Chowdhary et al (2002) Chandigarh and 3% by Brandonisio O et al (1999) S. Italy.

E.histolytica cysts were detected in 08% (18/234) in the present study. Other authors reported 13% by Cimerman S et al (1999) Brazil, 24.6% by Brandonisio O et al (1999) S. Italy, 16% by Chowdhary et al (2002) Mumbai and 3.9% by Mohammed Reza Zali et al (2004) Iran.

Table 5 E .coli was isolated from 36 cases (39%) ,followed by Shigellaflexneri21(25) and Pseudomonas aeruginosa from 21 cases (25%) each and Enterococcus faecalis from12case (14).

Table 06 Isospora belli and Strongyloides larvae were detected from 15case (38%)followed by Giardia lamblia& Pseudomonas aeruginosa 15(38%)and E. coli & E. histolytica from 09(23%) each

Table 07 shows antibiotic sensitivity pattern of Gram negative bacterial isolates. In present study 95% sensitivity was observed to Amikacin, Tetracycline, and Cefuroxime with all Gram negative isolates except to Pseudomonas aeruginosa. E.coli were totally resistant to Ampicillin.

Table 08 shows *Enterococcispp* was totally resistant to Ampicillin, Erythromycin, Cloxacillin and 100% sensitive to Cefperazone+ Sulbactam, Amikacin, Gentamycin and Cephalexin.

Table 09 parasites detected in control group are Giardia and Ascaris. Opportunistic parasites were not detected in control group

CONCLUSION

The present study included 300 HIV seropositive individuals with opportunistic infections. An attempt was made to identify/ isolate the etiological agents of opportunistic infections depending upon symptomatology and to correlate with CD4 cell count. Control group of HIV seronegative persons with similar symptomatology were also studied for the presence of various pathogens.

1. Out of 100 HIV seropositive patients studied 68% were males and 32% females. 86% individuals were between 21-40 years, which is the sexually active age group. 63% of them were from rural area and

belonged to low socioeconomic status (74%). 54% of individuals were illiterates .100% gave a history of heterosexual route of transmission.

2. 42% of individuals were with CD4 count 50-200/mm^3 , 29% with CD4 count < 50/ mm^3, 20% with CD4 count 200 - 500/mm^3, only 9% above > 500/ mm^3.
3. Infection of Isospors ,cryptosporidium ,cyclospora was significantly higher among HIV positive.

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