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Research Article

STUDY OF OPPORTUNISTIC INFECTION OF INTESTINAL PARASITIC AND BACTERIAL IN HIV /AIDS POSITIVE INDIVIDUALS AND CORRELATION WITH CD4 COUNT

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ABSTRACT

Objective: Intestinal parasities and bacterial predominant in HIV positive patient, CD4 cell count variation,
Material and Methods: study was conducted during January 2014 to July 2014 HIV positive cases ,microscopically , bacteriological culture, and CD4 cell count,
Results: 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 06(08%) each. *E .coli* was isolated from 12 cases (39%), followed by *Shigella flexneri*07(25) and *Pseudomonas aeruginosa* from 07 cases (25%) each and *Enterococcus faecalis* from04case (14).
Keywords: HIV/AIDS, CD4 cell count, opportunistic parasite and bacterial

INTRODUCTION

AIDS is caused by a retrovirus, HIV. 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. This has posed the greatest challenge to public health because of its emergence and pandemic spread.

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]. The consequence of CD4 T cell dysfunction are devastating because of the role it plays in human immune response, activation of macrophages, induction of functions of cytotoxic T cells , NK cells & B cells, secretion of a variety of soluble factors that induce growth and differentiation of lymphoid cells and affect hematopoietic cells.

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, hence these could be non – pathogenic in individuals with intact immune system (*Candida albicans*) or known pathogens presenting in a different way than usual in immunocompetent individuals (*Cryptococcus neoformans*) or

in the form of increased virulence, recurrence, multi drug resistance (*mycobacterium tuberculosis*) or atypical presentation (*dermatophytosis*)

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 HIV. Early 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 100 stools, samples were collected from100 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**, **FATHIMAINSTITUTE OF MEDICAL SCINCES, KADAPA**

Methodology:

1. Collection of samples

2. Direct Microscopy

A) Modified Ziehl-Neelsen staining for stool.

B) Saline mount & Iodine mounts for stool.

3. Isolation by Culture

a) NA, BA, Mac for sputum, stool,

b) Deoxycholate citrate agar, Wilson Blair, TCBS, Selenite F broth & Alkaline peptone water for stool.

4. Tests for Identification of isolates

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

Among hundred HIV seropositive individuals studied, 68% were male and 32% were females. 90.62% (29/32) of the females were in 21-40 years age group. 86.35% (57/68) of males were in 21-40 years age group. 86% (86/100) of individuals were between the age group 21- 40 years. 3.03 %(2/68) of males were in age group > 50 years & no females were present in this group (Table 1).

Out of hundred cases of HIV seropositive individuals studied, 63 individuals were from rural areas (63%) and 37 individuals were from urban areas (37%) (Table 2).

74% of HIV seropositive individuals from study group belonged to low socio economic status and 26% belonged to middle socioeconomic status (Table 3).

Table 1: Age and sex wise distribution of study group (n=100)

Sl. No	Age in years	Male	Age specific %	Female	Age specific %	Total
1.	10 -20	1	1.5	2	6.25	3
2.	21-30	27	40.90	20	62.5	47
3.	31-40	30	45.45	9	28.12	39
4.	41 50	8	12.12	1	3.12	9
5.	>50	2	3.03	-	-	2

Table 2: Residence pattern of study group (n=100).

No of cases	Rural	Urban
100	63	37

Table 3: Socio-economic status of study group (n=100).

Group	Number	%
Low economic status Rs <11,500 per annum.	74	74
Middle economic status Rs. 11,500-60,000 per annum.	26	26

Table- 4 shows the education status among 100 HIV seropositive individuals. 55.88% (38/68) of males and 50% (16.32) of females were illiterates. 16.17% (11/68) of males and 37.5% (12/32) of females had primary education .14.70% (10/68) of males and 9.37 %(3/32) of females had secondary education. 13.23% (9/68) of males and 3.12% (1/32) of females had college education.

Table -5 shows 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.

Table- 6 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 4: Literacy status of study group (n=100).

Education status	Male	%	Females	%	Total
Illiterates	38	55.88	16	50	54
Primary Education	11	16.17	12	37. 5	23
Secondary Education	10	14.70	3	9.37	13
College Education	9	13.23	1	3.12	10

Table 5: Occupational status of study group (n=100).

Sl. No	Occupation	Male	%	Female	%	Total
1.	Labourer	31	45.58	17	53.12	48
2.	Driver	16	23.52	-	-	16
3.	Business	4	5.88	-	-	4
4.	Agricultural	8	11.76	-	-	8
5.	Employee	6	8.82	1	3.12	7
6.	Unemployee	3	4.41	-	-	3
7.	House wives	-	-	14	43.75	14

Table 6: Mode of transmission (n=100).

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

Out of 100 seropositive individuals, 7.35% (5/68) of males and 12.5% (4/32) of females had CD_4 counts >500/mm^3. 20.58% (14/68) of males & 18.75% (6/32) of females had CD_4 counts 200 – 500/mm^3.44.11% (30/68) of males and 37.5% (12/32) of females had counts 50 – 200/mm^3. 27.94% (19/68) of males and 31.25% (10/32) of females had counts < 50/mm^3 (Table 7).

Total 100 Samples were collected. Out of 100 stool samples 78 (78%) were culture/smears positive (Table 8).

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* and *Giardia trophozoites* 06(08%) each (Table 9).

E .coli was isolated from 12 cases (39%) ,followed by Shigellaflexneri07(25) and Pseudomonas aeruginosa from 07 cases (25%) each and *Enterococcus faecalis* from 04case (14) (Table 10).

Isospora belli and *Strongyloides larvae* were detected from 05case (38%) followed by *Giardia lamblia*& *Pseudomonas aeruginosa* 05(38%) and *E. coli & E. histolytica* from 03(23%) each (Table 11).

Pseudomonas aeruginosa were 77.77% (9/12) sensitive to Amikacin, 58.33% (7/12) to *Gentamycin,* 25% (3/12) sensitive to Ciprofloxacin, 11.11% (1/9) sensitive to Cephalexin and 77.77% (9/12) sensitive to Cefuroxime and Tetracycline. E. coli totally resistant to Ampicillin, 100% sensitive to Amikacin ,Cefuroxime, Tetracycline and 66% (10/15) sensitive to Gentamycin, Ciprofloxacin and Cephalexin .Shigellaflexneri were 42% (3/7) sensitive to Ampicillin & Cephalexin and 100% (2/2) sensitive to Amikacin, Gentamycin Ciprofloxacin, Cefuroxime and Tetracycline (Table 12).

Enterococci spp was totally resistant to Ampicillin, *Erythromycin, Cloxacillin* and 100% sensitive to Cefperazone+ Sulbactam, Amikacin, Gentamycin and Cephalexin (Table 13).

Parasites detected in control group are Giardia and Ascaris. Opportunistic parasites were not detected in control group (Table 14).

Table 7: Sex wise pattern of CD4counts in HIV positive patients (n=100).

CD4	Male	%	Female	%	Total
>500/mm^3	5	7.35	4	12.5	9
200 –500/mm^3	14	20.58	6	18.75	20
50 – 200/mm^3	30	44.11	12	37.5	42
<50/mm^3	19	27.94	10	31.25	29

Table 8: Total number of samples collected -culture positivity (n=).

Sl. No	Samples	Total Number	Culture / smear Positive	%
1	Stool	100	78	78

Table 9: Parasites detected in stool samples (n=78)

Sl. No.	parasites	No. of cases	%
1.	*Isospora belli oocyst*	28	40
2	*cryptosporidium*	15	21
3	*Strongyloidesstercoralis larva*	12	17
4.	*Ascaris ova*	11	16
5.	*E. histolytica cyst*	06	08
6	*Giardia trophozoites*	06	08

Table 10: Bacterial isolates from stool samples (n=28).

Sl. No.	Parasites	No. of cases	%
1.	*E .coli*	12	39
2.	*Shigellaflexneri*	07	25
3.	*Pseudomonasaeruginosa*	07	25
4.	*Enterococcusfaecalis*	04	14

Table 11: Mixed bacteria and parasites detected from stool samples (n=13).

Sl. No.	Parasites	No. of cases	%
1.	*Isospora+ Strongyloides*	05	38
2.	*G.lamblia +Pseu.aeruginosa*	05	38
3.	*E. histolytica+ E. coli*	03	23

Table 12: 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	12	-	-	9	75	7	58.3	2	22.2	1	11.1	9	75	9	75
E .coli	15	R	-	15	100	10	66	10	66	10	66.6	15	100	3	100
Shigellaflexneri	7	3	42	7	100	7	100	7	100	3	42	7	100	7	100

(R=Resistant; — = not tested)

Table 13: Antibiotic sensitivity pattern of Gram positive bacterial isolates (n=45).

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	4	R	-	4	100	R	-	R	-	4	100	4	100	4	100

(R=Resistant)

Table 14: 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

DISCUSSION

Samples were collected 100 HIV seropositive individuals.. Control group of HIV seronegative patients with similar symptomatology were also studied for various pathogens.

Table 1 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.

Table 2 shows 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.

Table 3 shows 74% of individuals belong to low socioeconomic status. This observation coincides with findings of Singh A et al (2002) Manipal.

Table 4 & 5 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.

Table 6 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 7 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 8 shows the total number of samples collected from patients and the percentage of culture positivity., 78% (78/100) from stool samples,

In the present study Isospora 40 %(28/78) 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% (12/78) 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% (11/78) 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% (06/78) 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% (06/78) 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 10E .coli was isolated from 12 cases (39%) ,followed by Shigellaflexneri07(25) and Pseudomonas aeruginosa from 07 cases (25%) each and Enterococcus faecalis from04case (14).

Table 11 shows Isospora belli and Strongyloides larvae were detected from 05case (38%)followed by Giardia lamblia& Pseudomonas aeruginosa 05(38%)and E. coli & E. histolytica from 03(23%) each

Table 12 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 13 shows *Enterococcispp* was totally resistant to Ampicillin, Erythromycin, Cloxacillin and 100% sensitive to Cefperazone+ Sulbactam, Amikacin, Gentamycin and Cephalexin.

Table 14 parasites detected in control group are Giardia and Ascaris. Opportunistic parasites were not detected in control group

CONCLUSION

The present study included 100 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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