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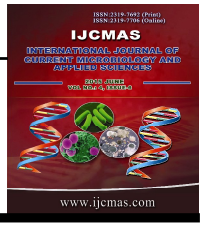

Original Research Article

Study of Opportunistic Infections in HIV-AIDS Patients and their Co-Relation with CD4+Cell Count

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Keywords

Opportunistic infections, CD4 cell counts, HIV

A B S T R A C T

The aim of this study to determine the spectrum of opportunistic infections in adult AIDS patients and correlate different opportunistic infections (OI's) with the $CD4^+$ cell count. A total of 159 patients were tested for spectrum of opportunistic infections. All the specimens were processed as per standard procedures to detect bacterial, fungal, parasitic and viral infections. $CD4^+$ counting of blood samples was done by Flow cytometry as per manufacturer's instructions (FACSCalibur, Becton- Dickinson, Immunocytometry system).Correlation of CD4 cell counts was done with the respective opportunistic infections. Male preponderance with male: female ratio i.e. 3.7:1 was seen. The young sexually active group (18-48 years) comprising of 93.7% of patients was affected. Among opportunistic infections, most common were bacterial (44.14%), fungal (32.43%), parasitic (16.21%) and viral infections (7.20%) of patients. Out of 222 events of OI's 26 %(58/222) were seen in $CD4^+$ count <50(cells/µl), 37.2% (83/222) were seen within 50-99 $CD4^+$ cells/ µl range followed by, 21.1% (47/222) in 100-149 CD4 cells/ µl while 15.2%(34/222) OI's were observed in 150-199 $CD4^+$ cells/ µl group and only 0.4% (1/222)OI's were seen in <200-500 $CD4^+$ cells/ µl group. Early diagnosis & prompt treatment of opportunistic infections is important before development of severe immunodeficiency to prevent serious and fatal outcome.

Introduction

Opportunistic infections are the manifestations caused by the pathogens which are usually not invasive but invade the body when its immunity is hampered because of one or the other reasons. It occurs in conditions like AIDS, various malignancies, steroid therapy, radiotherapy and diabetes. With the changing scenario of AIDS epidemic, a host of opportunistic infections add to the present endemic state of some already existing infections like tuberculosis .Opportunistic infections

account for the majority of the mortality and the morbidity in AIDS .The type of pathogen responsible for morbidity and mortality vary from region to region. The identification of such pathogen is very important to manage such cases.[1]

A person infected with HIV develops into a case of full blown AIDS by appearance of a variety of infections, labeled as opportunistic infections and malignancies. Common opportunistic infections seen in America and Europe are Pneumocystis carinii pneumonia (PCP), Cryptococcal meningitis, Cytomegalovirus (CMV), and Toxoplasmosis. But in developing countries of Africa, South, and south-east Asia, Mycobacterium tuberculosis ranks as the most common infection seen in the reported AIDS cases. [2]

According to latest report by NACO(2007), 65% cases of Tuberculosis, 57.5% of Candidiasis,36% of Cryptosporidiasis, 14% Herpes Zoster, 13% PCP, 9% bacterial pneumonia, 9% Cryptococcal meningitis, 3.8% Toxoplasmosis and Kaposi's sarcoma 0.17% have been found in India.[3]

Most of the opportunistic infections broadly correlate with CD4 lymphocyte count in majority of the cases.[4] HIV presently accounts for the highest number of deaths attributable to any single infective agent. India has an estimated 5.2million HIV-infected people. The threat to their life is not from virus alone. Opportunistic infections (OI's) and associated complications account for a considerable proportion of such mortality. Appropriate management of OI's is as important as antiretroviral therapy (ART) in preventing mortality and morbidity among HIV-infected persons.

With this background, present study was undertaken to study the spectrum of opportunistic infections in adult AIDS patients & to study the correlation of different opportunistic infections (OI's) with the $CD4^+$ cell count.

Materials and Methods

The prospective study was conducted in the Department of Microbiology, Government Medical College & Hospital (GMCH), Nagpur from May 2006 to October 2007 after approval from ethical committee.159 HIV positive patients either hospitalized or coming to ART clinic were included in the study.

CD4/CD3 enumeration: The CD4/CD3 enumeration was done using the single BD FACS count machine Becton Dickinson and company, USA by strictly following the manufacturer's instructions.

Depending on the patients clinical features various specimens were collected which included sputum, stool, CSF, oral swab, lymph node & skin scrapings.

Stool specimens were collected and examined microscopically using saline wet mounts. Lugol's iodine was used for the detection of ova, larva, trophozoites and cyst of intestinal parasites. Smears were examined by Modified acid fast staining for Cryptosporidium parvum, Isospora belli and other. Also bacteriological culture of stool was done for identifying bacterial infections.

Candidiasis was diagnosed by taking oral swab specimens and was cultured on Sabouraud's dextrose agar and suspected colony was identified by Germ tube test. Cryptococcal meningitis was diadgnosed by using India ink preparation of CSF and culture on SDA.

Herpes infection was diagnosed by taking impression smears from vesicular lesions

and by demonstrating multinucleated giant cells by Tzanck test using Giemsa stainor H&E.

Results and Discussion

In the present study, total 159 HIV positive AIDS patients were included. Out of these patients, 125 were males (78.6%) and 34(21.4%) were females. Male to female ratio was found to be 3.7: 1.Most of the patients (93.7%) belonged to 18-48(years) age group (Table No.1).

In this study total 222 opportunistic infections were found comprising of bacterial, fungal, parasitic & viral infections. Among different opportunistic infections, bacterial infections were seen in 44.14% patients, followed by fungal in 32.43%, parasitic in 16.21% & viral in 7.20% respectively. (Table No. 2)

In the present study total 222 events of OI's were found comprising of bacterial, fungal, parasitic and viral infections. Most of the OI's ie 26 %(58/222) were seen in $CD4^+$ count <50(cells/μl), 37.2% (83/222) were seen within 50-99 $CD4^+$ cells/ μl range followed by, 21.1% (47/222) in 100-149 CD4 cells/ μl while 15.2%(34/222) OI's were observed in 150-199 $CD4^+$ cells/ μl group and only 0.4% (1/222)OI's were seen in <200-500 $CD4^+$ cells/ μl group.

OI's association with $CD4^+$ count <50 (cells/μl)

In this group, 26 %(58/222) of opportunistic infections were seen which comprised of 43.1% (25/58) bacterial infections, 39.7% (23/58) fungal infections, 6.9% (4/58) parasitic infections and 10.3% (6/58) of viral infections.

Among bacterial infections, pulmonary tuberculosis and extra-pulmonary tuberculosis were seen with equal frequency i.e. 40% each (10/25), *Klebsiella pneumoniae* 12% (3/25), *Staphylococcus aureus* 4% (1/25) and *E. coli* 4 % (1/25).

In fungal infections, most common was Candidiasis 69.6 %(16/23) followed by *Cryptococcal meningitis* 21.7% (5/23), *Pneumocystis jiroveci* 4.3% (1/23) and *Trichophyton rubrum* 4.3 % (1/23).

Parasitic infections included *Cryptosporidium* 50% (2/4) followed by Isospora 25 % (1/4), *Strongyloides* 25% (1/4).

Viral infections included Herpes zoster 50% (3/6), *Molluscum contagiosum* 33% (2/6) and *Cytomegalovirus retinitis* in 16.7 % (1/6) patients.

OI's association with $CD4^+$ count 50-99 (cells/μl)

Most of the OI's ie 37.2% (83/222) were seen within 99-50 CD4 cells/ μl range which comprised of 45.8% (38/83) bacterial infections, 31.3% (26/83) fungal infections, 16.7%(14/83) parasitic infections and 6.0%(5/83) of viral infections.

In this group, among bacterial infections most common was pulmonary tuberculosis 47.4% (18/38) followed by extra-pulmonary tuberculosis 44.7% (17/38), *Klebsiella pneumoniae* 7.9% (3/38) and *Staphylococcus aureus* 2.6% (1/38).

In fungal infections, most common was Candidiasis 76.9 %(20/26) followed by *Cryptococcal meningitis* 19.2% (5/26) and *Trichophyton rubrum* 3.8% (1/26).

Parasitic infections included *Cryptosporidium parvum* 64.3% (9/14) followed by *Isospora belli, Strongyloides stercoralis, Hymenolepis nana, Entamoeba.*

histolytica and *Toxoplasma gondii* each 7.1% (1/14).

Viral infections included Herpes Zoster in 60% (3/5) and genital herpes in 40 % (2/5).

OI's association with CD4$^+$ count 100-149 (cells/µl)

In this group, 21.1% (47/222) of opportunistic infections were seen which comprised of 48.9% (23/47) bacterial infections, 31.9% (15/47) fungal infections, 17% (8/47) parasitic infections and 2.1% (1/47) of viral infections.

In this group, among bacterial infections most common was extra-pulmonary tuberculosis 47.8% (11/23) followed by pulmonary tuberculosis 26% (6/23), *Klebsiella pneumoniae* 8.7% (2/23) and *Streptococcus pneumoniae, Staphylococcus aureus, Escherichia coli* and Nonfermenter 4.3% (1/23) each.

In fungal infections, most common was Candidiasis 86.7% (13/15) followed by *Pneumocystis jiroveci* 13.3% (2/15).

Parasitic infections included *Cryptosporidium parvum* 75% (6/8) followed by *Strongyloides stercoralis*, *Hymenolepis nana* each 12.5% (1/8).

Viral infections included venereal warts only in one patient.

OI's association with CD4$^+$ count 150-199 (cells/µl)

In this group 15.2% (34/222) OI's were observed which consisted of 35.3% (12/34) bacterial infections, 23.5% (8/34) fungal infections, 29.4% (10/34) parasitic infections and 11.8 %(4/34) of viral infections.

In this group, among bacterial infections most common was pulmonary tuberculosis 50% (6/12) followed by extra-pulmonary tuberculosis 25% (3/12*), Klebsiella pneumoniae, Salmonella typhi and Shigella dysentriae* each 8.3%(1/12)each.

In fungal infections, only member was Candidiasis 100% (8/8).

Parasitic infections included *Cryptosporidium parvum* 60% (6/10) followed by *Strongyloides stercoralis, Isospora belli, Giardia lamblia and Entamoeba histolytica* each 10% (1/10).

Viral infections included Herpes zoster 75% (3/4) and venereal warts 25% (1/4).

OI's association with CD4$^+$ count 200-500 (cells/µl)

Only 0.4% (1/222) OI's were seen in 200-500 CD4$^+$ cells/ µl group. Only infection seen was of Candida species.

Acquired Immunodeficiency Syndrome (AIDS) is a pandemic of 21st century presenting with severe immunodeficiency in which patients present with symptoms of different opportunistic infections.

HIV presently accounts for the highest number of deaths attributable to any single infective agent. India has an estimated 5.2million HIV-infected people. The threat to their life is not from the virus alone. Opportunistic infections (OI's) and associated complications account for a considerable proportion of such mortality.

Thus it is very important to identify and start appropriate management of the offending agent at an earliest so that OI's can be managed appropriately to prevent mortality and morbidity among HIV-infected persons.

The present study of 159 HIV patients deals with wide spectrum of opportunistic infections and their correlation with $CD4^+$ cell counts.

The age of the cases ranged from 20 to 65 years. Majority of the patients (93.7%) belonged to 18-48 years age group which is similar to what has been reported byKumarasamy N et al[5], Singh A et al[1] and .

In the present study males (78.6%) outnumbered the females (21.4%).Male to female ratio was found to be 3.7:1 which is quite near to the ratio reported by Uzgare R et al[6] and Singh A et al[1].

In the study of opportunistic infections, we found bacterial infections as prominent opportunistic infection followed by fungal, parasitic and viral in decreasing order. Out of total 159 patients under study, 222 events of opportunistic infections were seen in the present study singly/ in mixed form.

Among bacterial infections, tuberculosis was found to be most common bacterial infection. It was seen in 50.9% of all the 159 patients out of which 49.4% were of pulmonary tuberculosis and 50.6% were of extrapulmonary tuberculosis. As far as tuberculosis is concerned we found almost equal distribution of pulmonary tuberculosis and extra-pulmonary tuberculosis in the study group with slightly more number of extra-pulmonary tuberculosis cases. Equal distribution of pulmonary tuberculosis and extra-pulmonary tuberculosis has also been observed byAyyagariet al[7] although in small number of cases.

Our findings are nearly similar to findings reported by Sunderam G. et al[8], Kumarasamy N et al[5],Misra SN et al[9], Veeranoot et al[10], M. Vajpayee et al[11], Singh A et al[1]and SanjeevSinha et al[12].

In the present study, *Klebsiella pneumoniae* was found to be most common pneumonia causing bacterial pathogen (8.2%). However relatively higher incidence (18.7%) of pneumonia due to *Klebsiella pneumoniae* has been documented by SanjeevSinha et al[12].Other organisms found to cause pneumonia in the present study were *Staphylococcus aureus, Escherichia coli and Streptococcus pneumoniae* which were also reported as common bacterial pathogens causing pneumonia by V VShailaja et al[13].

After bacterial pneumonia, other common bacterial infections found in present study are bacterial enteritis (3%) 1% each by *Salmonella typhi*, *Shigella dysenteriae* and Nonfermenter. However, slightly high incidence of Shigella (3.3%) and Salmonella enteritis (3%) has been reported by Kumar S Satheesh et al[14].

Fungal infections were the second common infections (45.3%) in all the 159 patients after bacteria infections in the present study. Among all the fungal infections Candidiasis was found to be the most common fungal infection (79.2%) in present study group which is in accordance with the range (12-93%) as reported by Greenspan.[15]

The high incidence of candidiasis in the present study can be explained by the fact that it is the second most common AIDS defining illness after tuberculosis in advanced stage of HIV infection in developing country like ours. Out of 57cases of candidiasis, disseminated candidiasis was found in 6 patients 10.5% (6/57) which is in accordance with reports by other workers (Kumar S Satheesh et al[14] and Sanjeev Sinha et al[16]).

In the present study second common fungal infection after candida was found to be Cryptococcal meningitis (13.9%) among all

fungal infections which is within the range (6-13%) reported by Murakawa et al[17].

Third common fungal infection found in present study was *Pneumocystis jiroveci* pneumonia (4.2%) among all fungal infections which is in the range (0.7-7%) as reported by Kumarasamy et al[18] in a recent study.

Higher incidence (10-33%) of pneumocystis jiroveci has also been reported by Patel AK et al [19],Malin Adam S. et al[20], Myoung-don Oh et al[21] and N. Usha Rani et al.[22]

Lower incidence found in present study as compared to above-mentioned reports may be due to difference in types of samples processed. In the present study, induced sputum samples were processed for demonstration of *Pneumocystis jiroveci* while other workers apart from processing sputum, have also used bronchoalveolar lavage, endobronchial brushings or even lung biopsy.

The disparity in the incidence of *Pneumocystis jiroveci* in present study from the others mentioned earlier may also be due to the fact that majority of the patients in present study group belonged to later stage of HIV infection at which chemoprophylaxis is already initiated as per NACO guidelines.

Besides Candidiasis, Cryptococcal meningitis and Pneumocystis jiroveci infections, Cutaneous ringworm infection (2.8%) by *Trichphyton rubrum* among all the fungal infections has been found in the present study group which is relatively less in comparison to the finding of Sivayathorn A et al[23].

After bacterial and fungal infections next common group of infections in the present study were found to be parasitic infections i.e. 22.6% of all the 159 patients. The most common parasitic infestation observed was *Cryptosporidium parvum* (63.9%). Agarwal A et al[24] has reported Cryptosporidium parvum in 81.8% of patients while 56.5% incidence of Cryptosporidium has been documented by Javid Sadraei et al [25] which is in accordance with findings of present study.

Next common parasite after Cryptosporidium inpresent study was *Strongyloides stercoralis*. Larvas of Strongyloides were seen in 11.1% of 36 cases of parasitic infestations. Lanjewar et al [26] has reported *Strongyloides stercoralis* in 9.1% as isolated and mixed infection.The findings in the present study are in accordance with Lanjewar et al[26] &Ghorpade et al[27].

Like other studies we also found Strongyloides stercoralis infection mixed with Cryptosporidium parvum and Isospora belli (Ghorpadeet al[27], Lanjewar et al [26] and Joshi M et al [28]).

After Cryptosporidium parvum and *Strongyloides stercoralis* next common parasitic infection in present study was Isospora belli (8.3%) among all the parasitic infections. The infections were also seen as mixed infection with Cryptosporidium parvum and *Strongyloides stercoralis*.The findings in present study are almost similar to 7.5% incidence reported byGulickSM et al.[29]

Esfandiari A et al[30] has reported 10% prevalence while 9.9% Isospora belli has been reported bySauda F.C. et al[31].Findings in the present study are nearly similar to the observations of these two workers.

Next common parasitic infestation in present study was *Hymenolepis nana* (5.6%) among all parasitic infections which was also seen

as mixed infection with Cryptosporidium, Isospora and *Strongyloides stercoralis*. Hymenolepis nana has been reported in 0.6% cases as mixed infection by Kumar S Satheeshet al[14].

Next common parasite infection in present study was Entamoeba histolytica (5.6%) among all parasitic infections. Findings in present study are nearly same to those reported by Esfandiari et al[30]. However, there is wide range (1-14.9%) of Entamoeba histolytica infection as reported by Mohandas et al [32] and Joshi M et al[28].

Though the Toxoplasma gondii has been described as one of the most common opportunistic infection in AIDS patients, in the present study we found only one case of cerebral toxoplasmosis (2.8%) which is in accordance with findings reported byPonniah P et al[33],Sanjeev Sinha et al [12] and SK Sharma et al [34].

In the present study, among all parasitic infections a single case of Giardia lamblia was found (2.8%) which is in accordance with percentages reported by Joshi M et al[28] (4.3%)and Gulick SM et al.[29](1.5%).

Among opportunistic infections, viral infections were seen in 10.1 % of all 159 patients in this study. In this group of viral infections, Herpes virus infection especially zoster was found to be most common Herpes infection (56.3%).

In the present study, Herpes zoster infections were seen in 5.7% of total 159 patients. The incidence is slightly less than that reported by Kielbauchet al[35] (8%) and Patel AK et al[19](9.6%).However higher incidence (11.2-20%) has been reported by Sivayathorn A et al[23] from Thailand,Myoung-don Oh et al[21]andKumarasamy et al[36].

Of total 16 viral infections cases, 2 were found to be having genital herpes in present study (12.5%) which is in accordance with herpes genitalis in 7.7% patients as reported byKumarasamy et al[36] and 10.9% by Sivayathorn A et al.[23]

The cases of *Molluscum contagiosum* and venereal warts each two in number (1.3% each) of total 159 patients were observed in present study. Biswas Jyotirmay et al[37] reported Molluscum contagiosum in 1.4% patients. Kumarasamy et al (2000) reported Molluscum contagiosum in 1.3% of patients. Findings in present study group are similar to those reported by BiswasJyotirmay et al[37] and Kumarasamy et al [36].

Incidence of genital warts in present study (1.3%) is also in accordance with Kumarasamy et al[36] observation (1.2%).

In the present study apart from Herpes simplex and Pox viruses, we found only one case of Cytomegalovirus retinitis (6.3%) among all viral infections though high range (15-20%) has been reported by foreign workers (Jacobson et al 1988b[38] and Drew et al 1988[39]).

Study byVeeranoot et al [10] reported slightly lower incidence (2%) of Cytomegalovirus infection in AIDS patients.

ThoughBiswasJyotirmay et al[37] found Cytomegalovirus ocular lesions in 21.4% in HIV infected patients but his study group comprised of only ocular lesions in HIV positive patients.

Corelation of opportunistic infections with CD4$^+$ cell count

As the immunodeficiency advances, HIV positive patient becomes susceptible to variety of opportunistic infections because of profound immunosuppression. There

have been many reports showing the correlation between $CD4^+$ cell count and occurrence of opportunistic infections in HIV patients. We also tried to correlate the same in the present study.

To correlate the opportunistic infection with $CD4^+$ cell count, for the convenience we divided the study cases into five groups based on CD4 cell count (cells/mm^3) i.e. <500-200, 199-150, 149-100, 99-50 and <50 cells/mm^3.It was observed in the present study that when $CD4^+$ count starts falling below 500 cells/mm^3, first indication of immunodeficiency is seen in the form of oral thrush however other opportunistic infections are seen when the severity of immunodeficiency increases as CD4 cell count becomes less than 200 cells/mm^3.The only infection seen in the CD4 count range(200-500) was Candida infection 0.4%(1/223). No other opportunistic infections were seen in this group in the present study. Merle A et al [40],Chien-Ching Hung et al[41]and NACO[42]also reported the similar findings in their studies. Whereas Crowe et al [43], Giri TK et al [44]andMerle A et al [45] reported the pulmonary tuberculosis to be the commonest opportunistic infection in $CD4^+$ cell count range (200-500). However we did not get a single case of tuberculosis in this range of CD4 cell count in the present study.

However when immunodeficiency further progresses and $CD4^+$ cell count falls below 200 cells/mm^3i.e. (199-150), the most common infections after Candidiasis were pulmonary tuberculosis 50%(6/12) followed by parasitic infection in 29.4% (10/34) cases. Parasitic infections were found in the form of Cryptosporidium parvum 60% (6/10) in this range of CD4 cell count. Reactivation of Herpes zoster was also observed in 75 % (3/4) cases.

Pulmonary tuberculosis has also been reported by M Korzeniewska-Kosela et al [46], Myoung-don Oh et al [21]andVeeranootet al[10].

M. Vajpayee et al [11]also reported tuberculosis as most common opportunistic infection followed by parasitic diarrhea and oral candidiasis at this level of $CD4^+$cell count(<200 cells/mm^3).

However Merle A et al [45], Theur CP et al [47]and M Whiteman et al[48] found extra-pulmonary tuberculosis to be common when CD4 cell count declines below 200 cells/mm^3.

Cryptosporidium were also reported as common parasitic pathogens in this CD4 count range (190-150) by Crowe et al [43], Wittner et al [49]andJavid Sadraeiet al[25].

Reactivation of Herpes zoster at CD4 cell count below 200 cells/mm^3 was also noticed byMyoung-don Oh et al[21]and Merle A et al.[40]

When $CD4^+$ cell count further drops to below 149 cells/ mm^3 i.e. in the group (149-100), the spectrum of opportunistic infections changes. At this level of $CD4^+$ cell count there were more number of cases of opportunistic infections 21.1%(47/223) observed in the present study. These infections comprised in the order of frequency i.e. Candidiasis followed by Cryptosporidium, extra-pulmonary tuberculosis and pulmonary tuberculosis. At this level, Pneumocystis jiroveci 13.3 % (2/15) were also noted. In this range of CD4 cell count extra-pulmonary tuberculosis dominated with addition of Pneumocystis jiroveci and bacterial pneumonia.

Singh Y.N. et al [50] also reported the Pneumocystis jiroveci whereas JanoffENet al[51] found Pneumococcal Pneumonia to be

common bacterial infection at this level of $CD4^+$ cell count.

Most of the reports by other workers did not classify the $CD4^+$ cell count in the range 100-149 cells/mm^3 as they had taken into consideration the $CD4^+$ cell count <200cells/mm^3 as a group in place of 149-100 cells/mm^3.

In the present study, with further increase in degree of immunodeficiency and simultaneous decrease in $CD4^+$ cell count i.e. below 100cells/mm^3 or in the group (99-50), tuberculosis both pulmonary and extra-pulmonary dominated the spectrum of opportunistic infections which was followed by Candidiasis, Cryptosporidium and Herpes zoster. At this level of immunodeficiency, two new infections, Cryptococcal meningitis and cerebral toxoplasmosis were seen. Similar findings have also been reported byCrowe et al.[43] Toxoplasma andCryptococcus infections have also been reported byFauci[52] and NACO. [42]

McCabe et al[53] has also reported Toxoplasma infection whereas Giri TK et al[44]has found Cryptococcal meningitis at this level of immunodeficiency i.e. ($CD4^+$cell count <100 cells/mm^3).

When severe immunodeficiency occurs i.e. $CD4^+$ cell count <50 cells/mm^3 almost all opportunistic infections become manifest at this terminal stage of AIDS. In this scenario, the existing infections are seen with increasing frequency and in disseminated form.

Table.1 Age & Sex wise distribution of the total patients (n=159)

Age group(years)	Male	Female	Total	Percentage
18-28	15	5	20	12.57%
29-38	73	21	94	59.11%
39-48	30	5	35	22.01%
49-58	6	3	9	5.66%
59 & above	1	0	1	0.62%
Total	125	34	159	100%

Table.2 Distribution of opportunistic infections

Infection	Number	Percentage
Bacterial	98	44.14%
Fungal	72	32.43%
Parasitic	36	16.21%
Viral	16	7.20%
Total	222	100%

Table.3 Correlation of OIs with CD4 cell counts

Type of OI's	$CD4^+$ count (cells/µl) range						
	Opportunistic pathogens		<50 n=37	50-99 n=52	100-149 n=36	150-199 n=27	<200-500 n=7
Bacterial	M.tuberculosis	Pulmonary	10	18	6	6	
		Extrapulmonary	10	17	11	3	
	Klebsiellapneumoniae		3	2	2	1	
	Staphylococcus aureus		1	1	1		
	Streptococcus pneumoniae				1		
	Salmonella typhi					1	
	Shigelladysentriae					1	
	Nonfermenter				1		
	Escherichia coli		1		1		
Fungal	Candidiasis		16	20	13	8	1
	Cryptococcus neoformans		5	5			
	Pneumocystis jiroveci		1		2		
	Trichophytonrubrum		1	1			
Parasitic	Cryptosporidium parvum		2	9	6	6	
	Strongyloides stercoralis		1	1	1	1	
	Isospora belli		1	1		1	
	Hymenolepis nana			1	1		
	Entamoeba histolytica			1		1	
	Giardia lamblia					1	
	Toxoplasma gondii			1			
Viral	Herpes zoster		3	3		3	
	Genital herpes			2			
	Molluscum contagiosum		2				
	Venereal warts				1	1	
	Cytomegalovirus retinitis		1				

In the present study with increasing bacterial infections along with both forms of tuberculosis, Candida and Cryptococcus, we also found Cytomegalovirus retinitis in 16.7 % (1/6) cases , Molluscumcontagiosum in 33.3%(2/6) and Pneumocystis jiroveci in 4.3%(1/23).

Cytomegalovirus infections have also been reported byFauci[52],Crowe et al[43]and NACO[42]at this level of $CD4^+$ cell count.

Merle A et al[40]has also reported Molluscum contagiosum at $CD4^+$ cell count < 50 cells/mm^3.

Pneumocystis jiroveci has also been found to occur at $CD4^+$ cell count <50 cells/mm^3byGiri TKet al.[44]
NACO [42]and Ballanl et al[54] have also reported Cryptococcal meningitis at $CD4^+$ cell count < 50/mm^3.

The male gender is the important factors in HIV-AIDS infection.Candidiasis as opportunistic infection is the first indication of immunodeficiency followed by tuberculosis and Cryptosporidium parvuminfection.There exists definite $CD4^+$ cell count correlation with the opportunistic infections in HIV-AIDS patients starting with Candidiasis, Pulmonary tuberculosis, Cryptosporidiosis, Herpes, Cryptococcal meningitis, Pneumocystis jiroveci pneumonia, Toxoplasma gondii and in terminal stage of immunodeficiency,bacterial pneumonia along with Molluscumcontagiosum and Cytomegalovirus retinitis in the order of increasing immunodeficiency.

Early diagnosis and prompt treatment of opportunistic infections is important before development of severe immunodeficiency to prevent serious and fatal outcome.

References

1. Singh A, Bairy I, Shivananda PG. Spectrum of opportunistic infections in AIDS cases. Indian journal of medical sciences. 2003; 57(1): 16-21.
2. National AIDS Control Organisation. Spectrum of opportunistic infections in AIDS cases in India. In: Specialist's training and reference module. New Delhi: 2000b.p. 107-11.
3. National AIDS Control Organisation, Ministry of Health and Family Welfare, Govt of India.Section 1. Introduction. In, Guidelines for prevention and Management of common opportunistic infections/ Malignancies among HIV-infected Adults and Adolescents. New Delhi: 2007a.p. 1-3.
4. National AIDS Control Organisation. Spectrum of opportunistic infections in AIDS cases in India. In: Specialist's training and reference module. New Delhi: 2000b.p. 107-11.
5. Kumaraswamy N, Solomon S, Jayaker Paul SA et al. Spectrum of opportunistic infections among AIDS patient in Tamil Nadu, India. Int J STD AIDS 1995; 6:447-9.
6. Uzgare R. Mode of transmission, of HIV in Mumbai (India) as per data collected in a private HIV/AIDS clinic. IntConf AIDS. 2000 Jul 9-14; 13: abstract no. TuPeC3369.
7. Ayyagari A, Sharma A.K. Prasad K. N. et al. Spectrum of opportunistic infections in HIV infected cases in a Tertiary Care Hospital. IJMM, 1999; 17 (2): 78 – 80.
8. Sunderam G, Reynard J, Me Donald, Maniati's T et al. Tuberculosis as a manifestation of acquired immunodeficiency syndrome (AIDS).JAMA 1986; 256:362-366.
9. Misra SN, Sengupta D, Satpathy SK.AIDS in India: recent trends in opportunistic infections. Southeast Asian J Trop Med Public health. 1998; 29(2):373-6.
10. Veeranoot N, Christopher, Lee, Queck K F, KhairulAnuar Abdullah. AIDS Related Opportunistic infections in Hospital Kula Lumpur. Jpn, J. Infect. Dis. 2003; 56: 187-192.
11. M. Vajpayee, S. Kanswal, P. Seth et al. Spectrum of Opportunistic Infections and Profile of $CD4^+$Counts among AIDS Patients in North India. Infection 2003; 31(5): 336-340.
12. SanjeevSinha and RandeepGuleria. Spectrum of Pulmonary Infections in

HIV Positive Patients: Indian Scenario. Chest. October 27, 2004.

13. V VShailaja, LA Pai, DR Mathur, V. Lakshmi. Prevalence of bacterial and fungal agents causing lower respiratory tract infections in patients with Human Immunodeficiency Virus infection. Indian Journal of Medical Microbiology 2004; 22(1):28-33.
14. Kumar S Satheesh, Ananthan S, Lakshmi P. Intestinal parasitic infection in HIV infected patients with diarrhoea in Chennai. Indian J Med Microbiol2002; 20(2):88-91.
15. Greenspan D. Greensan JS. : HIV related oral disease. Lancet 1996; 348:729-733.
16. SanjeevSinha and RandeepGuleria. Spectrum of Pulmonary Infections in HIV Positive Patients: Indian Scenario. Chest. October 27, 2004.
17. Murakawa G.J. et al. Cutaneous Cryptococcus Infection and AIDS. Arch. Dermatol, 1996; 132: 545.
18. Kumarasamy N, Vallabhaneni S, Flanigan TP, Mayer KH, Solomon S. Clinical profile of HIV in India. Indian J Med Res 2005; 121: 377-94.
19. Patel AK, Sheth JT, Vasa CV et al. HIV disease: clinical spectrum in state of Gujarat, India, IntConf AIDS. 1994 Aug 7-12; 10: 150 (abstract no. PB0028).
20. Malin Adams, Lovemore K.Z. Gwanzura, Kelin S et al: Pneumocystis carinii pneumonia in Zimbabwe. Lancet 1995; 346: 1258-61.
21. Myoung-don Oh, Sang Won Park, Hong Bin Kim et al. Spectrum of Opportunistic Infections and Malignancies in Patients with Human Immunodeficiency Virus Infection in South Korea, Clinical Infectious Diseases 1999; 29:1524 -1528.
22. N. Usha Rani, V.V.R Reddy, APrem Kumar et al. Clinical Profile of Pneumocystis Carinii Pneumonia In HIV Infected Persons. Ind journal of tuberculosis, 2000; 47:93.
23. Sivayathorn A, Srihra B, Leesanguankul W. Prevalence of skin disease in patients infected with human immunodeficiency virus in Bangkok, Thailand. Ann Acad Med Singapore. 1995; 24(4):528-33.
24. Agrawal A. Ningthouja S, Sharma D. et al. Cryptosporidium and HIV. J Indian Med Assoc 1998; 96: 276-77.
25. Javid Sadraei, Moshahid A. Rizviand U. K. Baveja. Diarrhea, $CD4^+$ cell counts and opportunistic protozoa in Indian HIV-infected patients. Parasitology Research, 2005; Vol.97 (4): 270-273.
26. Lanjewar D.N. Rodrigues C, Saple DG. et al. Cryptosporidium, Isospora and Strongyloides in AIDS. The Natl. Med. J. of India, 1996; 9(1): 17-19.
27. Ghorpade M.V. Kulkarni S.A., Kulkarni A.G. Cryptosporidium, Isospora and strongyloides in AIDS. Natl Med. J. India 1996; 9 (4): 201.
28. Joshi M, Chowdhary AS, Dalal PJ, Maniar JK. Parasitic diarrhoea in patients with AIDS. Natl Med J India, 2002 Mar-Apr; 15(2):72-4.
29. Gulick RM, Mellors JW, Havlir D et al. Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy. N Engl J Med 1997; 337: 734-9.
30. Esfandiari A. et al. Prevalence of enteric parasitic infection among HIV infected attendees of an inner city AIDS clinic. Cell. Mol.Biol.1995; 41 suppl. 1: 515-23.
31. Sauda FC et al. Prevalence of Cryptosporidium species and Isospora belli among AIDS patients attending Santos Reference center for AIDS, S ao Paulo, Brazil, J. Parasitol. 1993; 79(3): 454-56.

32. Mohandas, Sehgal R, Sud A, Malla N. Prevalence of intestinal parasitic pathogens in HIV – serpositive individuals in Northern India. Jon J Infect Dis. 2002; 55(3):83-4.
33. Ponniah P, Mallika M, Pankajalakshmi VV et al. Pattern of infection among HIV positive Indians. IntConf AIDS.1993 Jun 6-11; 9: 304 (abstract no. PO -B04 - 1015).
34. SK Sharma, TamilarasuKadhiravan, AmitBanga, et al. Spectrum of clinical disease in a series of 135 hospitalized HIV-infected patients from north India. BMC Infect Dis. 2004; 4: 52.
35. Kielbauch JA .Helicobacter associated bacteraemia and cellulitis in immunocompromised patients. Annals of internal medicine.1994; 125:90.
36. Kumarasamy Nagalingeswaran, Solomon Suniti, Madhivanan Purnima, Ravikumar Balakrishnan, Thyagarajan Sandras Panchatcharam, Yesudian Patrick.Dermatologic manifestations among human immunodeficiency virus patients in south India. International Journal of Dermatology. 2000. 39(3):192-195.
37. BiswasJyotirmay, Joseph Amala, RaizadaSeemant et al. Ophthalmic manifestations of human immunodeficiency virus (HIV) infection in India, Indian J Ophthalmol 1999; 47:87-93.
38. Jacobson M.A. Mills J. Serious cytomegalovirus disease in the Acquired Immunodeficiency Syndrome (AIDS).Clinical findings, diagnosis and treatment. Ann. Intern Med. 1988b; 108:585-94.
39. Drew W.L. Cytomegalovirus infection in patients with AIDS. J. Infect. Dis. 1988; 158:449-94.
40. Merle A. Sande, Paul A. Volberding.Editors. Dermatologic care of AIDS patients. In The Medical Management of AIDS 6th edit Publisher-WB Saunders company1999c, p185-194.
41. Chien-Ching Hung, Yun-Liang Yang, Tsai-Ling Lauderdale, et al .Colonization of Human Immunodeficiency Virus-Infected Outpatients in Taiwan with Candida Species. Journal of Clinical Microbiology, April 2005; 43 (4): 1600-1603.
42. National AIDS Control Organisation. Natural history and clinical manifestations of HIV/AIDS. In: Specialist's training and reference module. New Delhi: 2000c. p. 23-29.
43. Crowe SM, Carlin JB, Stewart KI, et al: Predictive value of CD4 lymphocyte numbers for development of Opportunistic infections and malignancies in HIV infected persons. J Acquir Immune DeficSyndr 1991; 4:770.
44. Giri TK, Pande I, Mishra NM et al .Spectrum of clinical and laboratory characteristics of HIV infection in northern India. Journal of Communicable Disease, 1995; 27(3):131-41.
45. Merle A. Sande, Paul A. Volberding, Editors. Tuberculosis in the HIV-infected patient.In .The Medical Management of AIDS 6th edit Publisher-WB Saunders company1999b, p353-359.
46. M Korzeniewska-Kosela, J M FitzGerald, S Vedal, et al. Spectrum of tuberculosis in patients with HIV infection in British Columbia: report of 40 cases. CMAJ 1992; 146(11): 1927–1934.
47. Theur CP, Hopewell PC, Elias D, et al: Human immunodeficiency virus infection in tuberculosis patients. J Infect Dis 1990 162:8.

48. M Whiteman, L Espinoza, MJ Post, et al. Central nervous system tuberculosis in HIV-infected patients: clinical and radiographic findings, American Journal of Neuroradiology.1995; 16(6): 1319-1327.
49. Wittner M, Tanowitz HB, Weiss LM: Parasitic infections in AIDS patients. Infect Dis Clin North Am 1993; 7:569.
50. Singh YN, Rattan A, Samanta Ray, JC. Pneumocystis carinii infection in AIDS in India. JAPI 1993; 41: 41-2.
51. Janoff EN, Breiman RF, Daley CL, Hopewell PC. Pneumococcal disease during HIV infection.Epidemiologic, clinical and immunologic Perspectives. Ann Intern Med 1992; 117: 314-24.
52. Fauci A.S., Lane H.C. Human Immunodeficiency virus (HIV) disease: AIDS and related disorders, in Harrison's Principles of Internal Medicine vol. 1. 16thedition. McGraw Hill, 2005, page -1076-1139.
53. McCabe R, Chirugi V: Issues in toxoplasmosis. Infect Dis Clin North Am 1993; 7:587.
54. Ballanl et al. Pulmonary Cryptococcosis during HIV infection. Rev. Mal. Respir. 1997; 14 (5): 365 - 370.