

OPPORTUNISTIC INFECTIONS IN RELATION TO CD4 COUNTS IN HIV SEROPOSITIVE PATIENTS IN A TERTIARY CARE HOSPITAL NORTH BIHAR

Pankaj Kumar¹, Ashwine Kumar Pandey¹, Ajay Kumar², R S Prasad³

Received : 10/01/2023
Received in revised form : 15/02/2023
Accepted : 27/02/2023

Keywords:

Opportunistic Infections, CD4 Counts, HIV Seropositive.

Corresponding Author:

Dr. Ajay Kumar,
Email: dr.ajay876@gmail.com

DOI: 10.47009/jamp.2023.5.3.70

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2023; 5 (3); 319-322



¹2nd Year Pg Student, Department of Microbiology, DMCH, Darbhanga, Bihar, India

²Assistant Professor, Department of Microbiology, DMCH, Darbhanga, Bihar, India

³Associate Professor, Department of Microbiology, DMCH, Darbhanga, Bihar, India

Abstract

Background: Human immunodeficiency virus (HIV) is a retrovirus, belonging to the lentivirus subgroup. It is the etiological agent of AIDS, which is a global pandemic. This study was therefore undertaken to evaluate the correlation between the patients CD4 counts and the presence of various OI's in patients with HIV. **Materials and Methods:** This prospective study was conducted in the Department of Microbiology, Darbhanga Medical College, Darbhanga, a tertiary care centre in North Bihar. It was an 18 months study conducted from July 2021 to December 2022. A total of 80 HIV-seropositive patients were estimated for OI and their CD4 counts. Universal precautions were adopted for processing every sample. All the samples were collected under aseptic conditions. The CD4 counts were measured by CD4 counter machine that works on the principle of flow cytometry. **Result:** In a total of 70 patients, 32 (45.7%) HIV positive patients screened were found to have OI. The following OI's were present on testing. Oral candidiasis (48.1%), Cryptosporidiosis (26.38%), Tuberculosis (9.52%), and CMV IgM, PCP, Isosporiasis and Cryptococcosis were (1.69%). Oral candidiasis was the most common infection found in these patients followed by opportunistic stool infection, Cryptosporidiosis. **Conclusion:** Our study concludes that the majority of OI's are present in patients with low CD4 counts. Therefore, careful search of these OI's in these patients is required.

INTRODUCTION

Human immunodeficiency virus (HIV) is a retrovirus, belonging to the lentivirus subgroup. It is the etiological agent of AIDS, which is a global pandemic. The virus is highly mutagenic in nature and the antigenic variations present, makes it survive in the human body. The mode of contact of the virus is through blood- and blood-stained body fluids. On entering the bloodstream, it acts primarily on the CD4 (helper/inducer) T lymphocyte. The binding of the virus to the receptor is by the envelope glycoprotein gp 120. The co receptor molecule is the CXCR 4 for T-cell-tropic HIV strains and CCR 5 for the macrophage – tropic strains.^[1] CD4 molecule is a 55 kDa glycoprotein, and it is found predominantly on helper T lymphocytes and are also expressed on monocytes, macrophages and dendritic or langerhans cells. HIV envelope glycoprotein 120 (gp 120) binds to a portion of major histocompatibility complex binding site of CD4 molecule. When CD4 counts fall to <200 cells/ μ L, these patients become susceptible to opportunistic infections (OI).^[2] HIV infection leads

to low levels of CD4 counts making the body more susceptible to OI. This leads to increased morbidity and mortality of the patients, which is actually due to the OI rather than HIV itself.^[3] When CD4 T-cell counts decline below a critical level of 200 cells/ μ L, the cell-mediated immunity gets declined, and OI's appear.^[4] Therefore, it is important to identify the specific pathogen to manage such patients.^[5] Currently, no vaccines for HIV/AIDS have been approved for use, with many clinical trials still undergoing.^[6] This study was therefore undertaken to evaluate the correlation between the patients CD4 counts and the presence of various OI's in patients with HIV.

Aims and Objectives

To screen HIV seropositive patients for OI's in relation to their CD4 counts in a tertiary care hospital in North Bihar

MATERIALS AND METHODS

This prospective study was conducted in the Department of Microbiology, Darbhanga Medical College, Darbhanga, a tertiary care centre in North

Bihar. It was an 18 months study conducted from July 2021 to December 2022. A total of 80 HIV-seropositive patients were estimated for OI and their CD4 counts. Universal precautions were adopted for processing every sample. All the samples were collected under aseptic conditions. The CD4 counts were measured by CD4 counter machine that works on the principle of flow cytometry. A fresh 2 ml venous ethylenediaminetetraacetic acid sample was processed for the same. Sputum and bronchoalveolar lavage (BAL) samples were tested for tuberculosis, and *Pneumocystis carinii* pneumonia (PCP). To diagnose tuberculosis, preferably early morning expectorated sputum sample was collected in a sterile wide-mouthed universal container. The sample was subjected to concentration by using Petroff's method by using standard microbiological techniques.^[7] The concentrated sample was stained by Ziehl Nelson technique and cultured on Lowenstein Jensen media. Positive growth was confirmed by performing biochemicals – Tween 80 hydrolysis and Nitrate reduction test. To diagnose PCP, sputum sample, preferably induced sputum sample after 3% normal saline nebulisation and/or BAL samples were subjected to staining by Gomori's silver methanamine stain by standard microbiological techniques.^[8] The oocyst of *Pneumocystis carinii* (jirovecii) was seen as brown to black colored spherical structures, 8–10 mm in size against a green background. Oral candidiasis was detected by potassium hydroxide (KOH) preparation. Oral scrapings were taken for diagnosing the same, present as white mucosal patches. The material was put for digestion in 10% KOH, and wet mount smears were made to see oval budding yeast-like cells. The culture was done on Sabrauds dextrose agar with and without antibiotic at 37° and 22°. A Gram-staining was done from the growth to see Gram-positive budding yeast-like cells. Standard microbiological techniques were followed for the same.^[9] The stool OI's like Cryptosporidiosis, Isosporiasis and Cyclosporiasis were diagnosed by modified Ziehl Nelson staining. The stool samples were collected in a wide mouthed universal

container. Direct smears and smears after ether concentration method were prepared using standard microbiological techniques.^[10] Acid-fast pink colored oocyst was to be seen with respective structures and sizes.^[11] Serum samples were tested for Toxoplasmosis and Cytomegalovirus (CMV) infection for both acute (immunoglobulin M [IgM]) and chronic stage (immunoglobulin G [IgG]). The test was performed by enzyme-linked immunosorbent assay. The sensitivity and specificity of the both the kits were >98%. Cryptococcosis was detected by processing the cerebrospinal fluid (CSF) sample. India ink preparation was made to demonstrate negative staining of the capsulated organism.^[12] A latex agglutination test was done using cryptococcal antigen in CSF using Pastorex™ Cryptoplus 61747 Agglutination technique to detect capsular polysaccharide. It is a qualitative and semi – quantitative test that uses a simple agglutination technique to detect the capsular polysaccharide of *Cryptococcus neoformans*, glycuronoxylomannan (GXM), in biological fluids. This test uses latex particle coated with an anti – GXM monoclonal antibody. The major component of the capsule is GXM. The agglutination resulting from the reaction between the particles and GXM is visible to the naked eye. The sensitivity limit is 50 ng/ml of serum.

RESULTS

In a total of 70 patients, 32 (45.7%) HIV positive patients screened were found to have OI, [Table 1]. There were 16.3% patients presenting with infections having a CD4 count below 200 cells/μL. There were 6.0% patients with CD4 counts between 200 and 499 cells/μL and 0.94% patients with CD4 counts above 500 cells/μL. The following OI's were present on testing. Oral candidiasis (48.1%), Cryptosporidiosis (26.38%), Tuberculosis (9.52%), and CMV IgM, PCP, Isosporiasis and Cryptococcosis were (1.69%) [Table 2]. Oral candidiasis was the most common infection found in these patients followed by opportunistic stool infection, Cryptosporidiosis.

Table 1:

Total	Opportunistic infection
70	32

Table 2:

Opportunistic infection	Percentile
Oral candidiasis	48.1
Cryptosporidiosis	26.38
Tuberculosis	9.52
PCP	1.69
Isosporiasis	1.69
Cryptococcosis	1.69
CMV IgM,	1.69

DISCUSSION

In our study, out of the total 70 patients, 32 HIV positive tested (45.7%) had OI. The patients in our study were hospitalized patients and would have been on antiretroviral treatment and prophylactic therapy. Therefore, the number of patients presenting in our study with OI's are less in number. Cases that are newly diagnosed or taken directly from the community may probably present with decreased immunity, making the patients more vulnerable to OI. Among these positive patients presenting with OI's majority had a CD4 count of below 200 cells/μL that is, 16.3%. This is in accordance to a study by Kulkarni et al. who showed that the majority of the patients presenting with OI had a CD4 count of below 200 cells/μL.^[13] Dantie et al. also concluded that the prevalence of OI was found to be highest in patients with a CD4 count of <200 cells/μL and that there was a strong association between the two parameters.^[14] Oral candidiasis was the most common infection found in these patients followed by opportunistic infection, Cryptosporidiosis in the stool. In a study by Chakraborty et al. Oral candidiasis was the commonest OI present in 32% patients with CD4 counts of <200 cells/μL.^[15] Gableri et al. also found oral candidiasis to be the most common OI in patients with CD4 count of <200 cells/μL.^[16] In a study by Kulkarni et al. on 137 HIV patients, Cryptosporidiosis was the most commonly observed intestinal stool parasitic infection (54%). These patients also presented with CD4 counts of <200 cells/μL.^[13] This is in accordance to a study done by Tuli et al. Who showed maximum parasitic stool isolation in patients with CD4 count of <200 cells/μL.^[17] In a study by Dhungel et al. in Nepal, tuberculosis (30%) and oral candidiasis (14%) were the most common OI's detected.^[18] Studies have shown that there is a 5% incidence of tuberculosis found in HIV-infected patients.^[19] In our study, tuberculosis was found in less number of cases. This might be due to the endemic nature of the disease in our country and prior treatment by antitubercular drugs. *P.carinii* is rarely documented in India.^[20] In our study also the prevalence of PCP was less. In India, the incidence of 5% was reported by Lanjewar et al,^[21] and NACO,^[22] had reported an incidence of 4%, in India. Since the majority of the patients were hospitalized and presented with low CD4 counts prophylactic septran therapy given would have been protective in nature. HIV coinfection with cryptococcosis is also prevalent in patients with low CD4 counts.^[14] In our study, a low prevalence was found due to less number of positive cases and presenting illness of the patient. Acute infection with CMV was found to be less prevalent in our study (1.69%). As compared with the study by Akinbami et al., where a prevalence of 6.6% was found in patients with low CD4 counts.^[23]

CONCLUSION

Our study concludes that the majority of OI's are present in patients with low CD4 counts. Therefore, careful search of these OI's in these patients is required. CD4 counts can act as a reliable disease prognostic marker. In patients with CD4 counts of <200 cells/μL, the need for monitoring, prophylaxis and cure of OI's is required.

REFERENCES

1. Ananthnarayan R. Human immunodeficiency virus: AIDS. In: Paniker CK, editor. Text Book of Microbiology. 7th ed. Chennai: Orient Longman Private Ltd.; 2005. p. 582-98.
2. Muller V, Mocroft A, Reiss P, Katlama C, Papadopoulos AI. Relations among CD4 lymphocyte count nadir, Antiretroviral therapy and HIV – 1 disease progression: Results from the EUROSIDA study. *Ann Intern Med* 1999;130:570-7.
3. Noor R, Morsalin M, Chakraborty B. Reduction of CD4 count induces opportunistic infections in people living with HIV (PLHIV). *Bangladesh J Med Sci* 2014;13:285-91.
4. Cunningham AL, Donaghy H, Harman AN, Kim M, Turville SG. Manipulation of dendritic cell function by viruses. *Curr Opin Microbiol* 2010;13:524-9.
5. Ayyaari A, Sharma AK, Prasad KN, Dhole TN, Kishore J, Choudhary G. Spectrum of opportunistic infection in Human immunodeficiency virus (HIV) infected cases in tertiary care hospital. *Indian J Microbiol* 1999;17:78-80.
6. Preventive HIV Vaccines, AIDS info. (online). Available from: [http:// www.aidsinfo.nih.gov/vaccines](http://www.aidsinfo.nih.gov/vaccines). [Reviewed on 2006 May].
7. Watt B, Rayer A, Harris G. Mycobacterium. In: Collee JG, Fraser AG, Marrison BP, Simmons A, editors. Mackie and Mc Cartney Practical Medical Microbiology. 14th ed. New Delhi: Churchill Livingstone; 2012. p. 329-41.
8. Lumsden WH, Burns S, Mcmillan A. Protozoa. In: Collee JG, Fraser AG, Marrison BP, Simmons A, editors. Mackie and Mc Cartney Practical Medical Microbiology, 14th ed. New Delhi: Churchill Livingstone; 2012. p. 736-50.
9. Milne LJ. Fungi. In: Collee JG, Fraser AG, Marrison BP, Simmons A, editors. Mackie and Mc Cartney Practical Medical Microbiology. 14th ed. New Delhi: Churchill Livingstone; 2012. p. 695-717.
10. Lumsden WH, Burns S, Mcmillan A. Protozoa. In: Collee JG, Fraser AG, Marrison BP, Simmons A, editors. Mackie and Mc Cartney Practical Medical Microbiology. 14th ed. New Delhi: Churchill Livingstone; 2012. p. 721-54.
11. Chatterjee KD. Parasitology: Protozoology and Helminthology. 13th ed. New Delhi: CBS Publishers and Distributors Pvt. Ltd.; 2009. p. 135.
12. Milne LJ. Fungi. In: Collee JG, Fraser AG, Marrison BP, Simmons A, editors. Mackie and Mc Cartney Practical Medical Microbiology. 14th ed. New Delhi: Churchill Livingstone; 2012. p. 698.
13. Kulkarni SV, Kairon R, Sane SS, Padmawar PS, Kale VA, Thakar MR, et al. Opportunistic parasitic infections in HIV/AIDS patients presenting with diarrhea by the level of immunosuppression. *Indian J Med Res* 2009;130:63-6.
14. Dantie D, Yismaw G, Woldeyohannes D, Angaw B. Common opportunistic infections and their CD4 cell correlates among HIV– infected patients attending at antiretroviral therapy clinic of Gondar University Hospital, Northwest Ethiopia. *BMC Res Notes* 2013;6:534.
15. Chakraborty N, Mukherjee A, Santra S, Sarkar RN, Banerjee D, Guha SK, et al. Current trends of opportunistic infections among HIV-seropositive patients from Eastern India. *Jpn J Infect Dis* 2008;61:49-53.
16. Gabler IG, Barbosa AC, Velela RR, Lyon S, Rosa CA. Incidence and anatomic localization of oral candidiasis in patients with AIDS hospitalized in a public hospital in Belo Horizonte, MG, Brazil. *J Appl Oral Sci* 2008;16:247-50.
17. 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.
18. Dhungel BA, Dhungel KU, Easow JM, Singh YI. Opportunistic infection among HIV seropositive cases in Manipal Teaching Hospital. Pokhara, Nepal. *Kathmandu Univ Med J* 2008;6:335-9.
 19. Khedkar DT, Chitnis UB, Bhawalkar JS, Mamulwar MS. RNTCP – Evolution, achievements and challenges. *Med J DY Patil Univ* 2019;7:5-12.
 20. Singh A, Bairy I, Shivananda PG. Spectrum of Opportunistic infection in AIDS. *Indian J Med Sci* 2020;57:16-21.
 21. Lanjewar DN, Duggal R. Pulmonary pathology in patients with AIDS: An autopsy study from Mumbai. *HIV Med* 2021;2:266-71.
 22. NACO. HIV/AIDS surveillance in India. In: *Specialist's training and reference module*. New Delhi: NACO; 2022. p. 12-7.
 23. Akinbami AA, Rabiou KA, Adewunmi AA, Wright KO, Dosunmu AO, Adeyemo TA, et al. Seroprevalence of cytomegalovirus antibodies amongst normal pregnant women in Nigeria. *Int J Womens Health* 2023;3:423-8.