OPEN ACCESS
Saudi Journal of Pathology and Microbiology
Abbreviated Key Title: Saudi J Pathol Microbiol
ISSN 2518-3362 (Print) |ISSN 2518-3370 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: http://scholarsmepub.com/sjpm/

Original Research Article

Profile of Opportunistic Infections with CD4 Count in HIV Seropositive Cases at A Tertiary Care Centre

Dr. Ravikanti[1], MD., Dr. Rajeshwari K[2*], MD., Dr. Sunitha.B.R[3]. MD., Dr. G. Vishwanath[4], MD

[1]Assistant Professor, Department of Microbiology, Gulbarga Institute of Medical Sciences, Gulbarga, 585105. Karnataka, India
[2]Assistant Professor, Department of Pathology, Gulbarga Institute of Medical Sciences, Gulbarga, 585105.Karnataka, India
[3]Professor, Department of Microbiology, J.J.M. Medical College, Davangere, 577004. Karnataka, India
[4]Professor & Head, Department of Microbiology, J.J.M. Medical College, Davangere, 577004 Karnataka, India

DOI:10.21276/sjpm.2019.4.8.4 | **Received:** 28.05.2019 | **Accepted:** 09.07.2019 | **Published:** 20.08.2019

***Corresponding author:** Dr. Rajeshwari K

Abstract

***Introduction*:** AIDS is characterized by a number of opportunistic infections which are responsible for high morbidity and mortality. The spectrum and distribution of opportunistic infection (OIs) in AIDS patients is due to viral cytopathology and are secondary to the failure of both cellular and humeral response with CD4 count of <200 mm^3 leads to morbidity and mortality. ***Material and Methods*:** This is a descriptive and analytical study. Incidence of clinically and laboratory confirmed cases of opportunistic infections in HIV patients are recorded, during the one year period from June 2017- May 2018. Samples of these patients processed for CD4 counts, to assess the immune status. ***Results*:** 500 HIV seropositive patients with OI were studied. Out of these 308 (61.6%) patients had bacterial infections, of which TB (56%) was predominant infection with CD4 count <200mm^3. 85 (17%) patients had viral infections, of which common was Herpes zoster 72 (14.4%). 65 (13%) patients had fungal infections of which common was oral candidiasis 46 (9.2%) with CD4 count <100 mm^3. 42 (8.4%) patients had parasitic infections, of which common was chronic diarrhea 40 (8%) with CD4 count <100mm^3. Commonly observed risk group was in heterosexual group (86.8%). ***Conclusion*:** Our study helps the programme manager to plan appropriate strategies for the investigation and treatment of common OIs as a part of management programme for HIV infected populations. It helps them for early diagnosis and manage the patients.

Keywords: HIV infected patients, opportunistic infections, Seropositive, Tuberculosis.

INTRODUCTION

AIDS is an emerging pandemic viral infectious disease caused by Human Immunodeficiency Virus, which has posed the greatest challenge to public health in modern world. Clinical manifestations in HIV infections are primarily due to viral cytopathology and are secondary to the failure of both cellular and humoral immune response.[1,2,3,4,5]

Opportunistic infections with low CD4 counts influence the morbidity and mortality due to HIV infections.[1,3,5,6] Patients with CD4 counts >200 /mm^3 are 6 times more likely to develop opportunistic infections compared to those with CD4 counts of > 350/mm.[3]

In India, Tuberculosis is the most commonly reported opportunistic infection with CD4 cells >200 / mm^3.[2,3] Other commonly reported opportunistic infections among HIV infected are Oral Candidiasis, Herpes zoster, Cryptococcal Meningitis, Cerebral Toxoplasmosis and Cytomegalovirus Retinitis with CD4 counts < 200 /mm[3.6,10]

The prevalence of intestinal parasitic infections remains significant in HIV infected patients with low CD4 counts, i.e <100 /mm^3.The high incidence of commonly reported opportunistic infections with low CD4 counts in Indian HIV infected individuals highlights the need for early screening and also the need to increase awareness in health care providers in order to improve decisions regarding prophylaxis for prevention and appropriate therapeutic interventions.[10,11,12]

To determine role of CD4 decline and the incidence of opportunistic infections, CD4 counts as a clinical score serve as both an alarm for timing of prophylaxis and a guide for therapeutic intervention.[2,3,6,8]

It is also documented that types of opportunistic infections is profoundly influenced by geography, prevalence of infectious diseases in particular region, nutritional, socioeconomic conditions and other factors.[1,2,9,10] Therefore this study will be conducted to evaluate the correlation between CD4 counts in HIV infected patients with onset of specific opportunistic infections.[1,2,10,12]

Aims of study

- To document the spectrum of opportunistic infections in various age groups of HIV/AIDS patients and to note the CD4 counts among the group

MATERIALS AND METHODS

Source of data:

This was a prospective study involving proven cases of HIV/AIDS with signs and symptoms of opportunistic infections attending the outpatient department or admitted to Hospital during the one year period, from June 2017 to June 2018.

Sample size:

Five hundred HIV/AIDS seropositive patients with signs and symptoms of OIs, clinically, radiologically and diagnostically proven cases. Informed consent was taken from all patients during the study.

METHOD OF COLLECTION OF DATA

Inclusion criteria

- Confirmed HIV seropositive cases seeking medical care for signs and symptoms of opportunistic infections like PUO, Fungal infections, [Candidiasis, Cryptococcosis, Pneumocystosis, Histoplasmosis, Coccidiodosis], Herpes zoster and H.simplex, Pulmonary and Extrapulmonary Tuberculosis /Pneumocystis carinii, Persistent diarrhoea [more than one month], Lymphadenopathy, Refractory anemia, Idiopathic Thrombocytopenia.

Exclusion Criteria

- HIV seropositive individuals already on antiretroviral therapy, asymptomatic partners and children of HIV seropositive individuals, HIV seropositive individuals detected during routine ANC checkup, pre-operative, pre-employment and pre-insurance screening.

CD4 cell count

With strict aseptic precautions, 3ml of venous blood sample was collected by venepuncture using EDTA vacutainer and processed by flow cytometry, according to the standard protocol supplied by the manufacturer. (PARTEC IVD FLOW CYTOMETER machine, by Partec Gmbh. Am Flugplatg 13. D-02828 Gorlitz. Germany).

Principle

The mouse monoclonal antibody MFM-241 recognizes the human CD4 antigen, a transmembrane glycoprotein (55 kDa) of the immunoglobulin supergene family, present on a subset of T-lymphocytes ("helper/inducer" T-cells) and also expressed at a lower level on monocytes, tissue macrophages and granulocytes. Approximately 20-60% of human peripheral blood mononuclear cells as well as a subpopula-tion of monocytes but with a weaker signal are stained The antibody has been studied at the 8th International Workshop on Human Cell Differentiation Molecules HCDM (former HLDA VIII), May 2006, Quebec, Canada. CD4 is the primary cellular receptor for the human immunodeficiency virus (HIV).

Flow Cytometric Analysis

CD4-PE fluorescence can be analysed on a Partec Flow Cytometer with an excitation light source of 488 nm or 532 nm (blue or green solid state laser). To count CD4+ T-cells transfer the test tube with 84ftul of the ready prepared blood sample (see Method) to the Partec counting results will be displayed automatically as CD4+ T-cells per µl whole blood.

Method

- 20 µl whole blood (EDTA as anticoagulant) were taken in a Partec test tube.
- 20 µl of CD4 mAb PE then added and mixed gently, later incubated for 15 minutes at room temperature protected from light.
- 800 µl of no lyse buffer is added to Partec test tube and shaken or vortexed gently.
- Blood samples were then analyzed on a Partec device by aspiration and results displayed on screen were noted.

All clinical, radiological and laboratory data available, which includes baseline investigations in all HIV seropositive individuals, screening and confirmation for HIV infections available [Strategy III WHO] were recorded & documented in standard proforma for later analysis.

Diagnosis of opportunistic infections (OI)

Opportunistic infections in HIV patients were diagnosed as per the criteria fixed by center for disease control and prevention (CDC) [13].The HIV patients attending the outpatient department, depending upon the system and organ involvement, the specimens were collected. It included swabs, pus, sputum, CSF, blood, lymph node aspirates and stool was collected and subjected to special stains, microscopy, culture, serological tests and ancillary imaging techniques. The special stains includes grams stain, Zeihl-Nelson (Zn), Modified acid fast staining for cryptosporidium, silver methenamine staining for Pneumocystis jiroveci and Indian ink staining for cryptococcu. The culture includes blood agar, Mac Conkey agar, chocolate agar, Lowenstein-Jensen medium and Sabourad's dextrose

agar and BHI broth depending on the specimen. The growth on the media was subjected to a set of standard biochemical tests as per CLSI guidelines. The isolate was identified and reported.

Statistical Analysis

The collected data was tabulated, analyzed and subjected for statistical analysis using SPSS 19.0. Results are presented as range for quantitative data and number and percentage for qualitative data.

RESULTS

The present study was carried out on 500 HIV seropositive patients with signs and symptoms of opportunistic infections attending Hospital, ART center, over a period of one year, to know the incidence of bacterial, viral and fungal, parasitic infections and their correlation with CD4 count.

In the present study 500 HIV seropositive patients with opportunistic infections were included of which of which majority of the patients were males accounting for 55.2% of the cases with male to female ratio of 1.2:1.(table 1). The most common age group presentation in our study is between 31-40yrs accounting for 37.8% of the cases (table 2).

Table-1: Sex distribution of cases

Sex	NO.OF CASES	PERCENTAGE
Male	276	55.2%
Female	224	44.8%
TOTAL	**500**	**100.0%**

Table-2: Age distribution of cases

AGE	NO.OF CASES	PERCENTAGE
<20	35	7.0%
21-30	76	15.2%
31-40	189	37.8%
41-50	143	28.6%
51-60	39	7.8%
>60	18	3.6%
TOTAL	**500**	**100.0%**

The most common risk factor observed in our study is heterosexual route of transmission accounting or 86.8% of the cases (table 3).

Table-3: Distribution of risk factors among cases

RISK FACTOR	NO.OF CASES	PERCENTAGE
Heterosexual	434	86.8%
Msm	9	1.8%
Injecting Drug Use	2	0.4%
Blood Transfusion	12	2.4%
Mother To Child	22	4.4%
Probable Unsafe Injection	9	1.8%
Commercial Sex Work	9	1.8%
Truck Driver	3	0.6%
TOTAL	**500**	**100.0**

According to WHO, in our study, we observed that most of the HIV seropositive cases with opportunistic infections showed increased prevalence of grade – 3 of WHO grading system, accounting for 55.4% of the cases (table 4).

Table-4: Distribution of cases according to WHO grading

WHO GRADING	NO.OF CASES	PERCENTAGE
1	6	1.2%
2	95	19.0%
3	277	55.4%
4	122	24.4%
Total	**500**	**100.0%**

In the present study, occupation wise distribution of the cases in HIV seropositive with opportunistic infections showed maximum cases belonging to agricultural labourer accounting for 31.4 % of the cases. (Table 5)

Table-5: Occupation wise distribution of cases

Occupation	No.Of Cases	Percentage
Agricultural Labourer	157	31.4%
Non Agricultural Labourer	102	20.4%
Domestic Servant	3	0.6%
Skilled Worker	14	2.8%
Semi-Skilled Worker	4	0.8%
Petty Buisiness/Small Shop/Self Employed	28	5.6%
Service(Govt/Pvt)	20	4.0%
Student	20	4.0%
Truck Driver/Helper	10	2.0%
Local Transport Worker	19	3.8%
Hotel Staff	7	1.4%
Agricultural Cultivator/Land Holder	11	2.2%
Unemployed	11	2.2%
Retired	10	2.0%
House Wife	84	16.8%
Total	500	100.0%

In our study, out of 500 cases of HIV seropositives majority were bacterial infections accounting for 61.6% of the cases. Among the bacterial infections, the most common presentation of the case

observed was pulmonary tuberculosis with X- ray positive accounting for 14.8% of the total opportunistic infections cases with mean CD4 count <200 mm^3.

Next common opportunistic infections observed was viral infection accounting for 17 % of the cases. Among the viral infections, the most common infection seen was Herpes Zoster accounting for 14.4% of the total opportunistic infections cases with mean CD4 count <100 mm^3.

The other common opportunistic infections seen is fungal infection accounting for 13% of the cases. Among the fungal infections, the most common infection seen was Oral candiasis accounting for 9.2% of the total opportunistic infections cases with mean CD4 count <100 mm^3.

The other opportunistic infections seen is parasitic infection accounting for 8.4 % of the cases. Among the parasitic infections, the most common infection seen was intestinal parasitic infection presenting with chronic diarrhea with accounting for 8.0% of the total opportunistic infections cases with mean CD4 count <100 mm^3.

Out of 42 cases, 40 (95.2%) cases were chronic diarrhea and each case had malaria and toxoplasmosis with CD4 count <100mm^3. (table 6).

Table-6: Distribution of opportunistic infections among cases with mean CD4 count

Sl. No	DISEASES	NO.OF CASES	MEAN CD4 COUNT
1	**Bacterial Infections**	**308(61.6%)**	
a.	TB Lymphnode	30(6%)	170.2
b.	TB Memningitis	12(2.4%)	65.6
c.	TB Spine	2(0.4%)	161
d.	TB Abdomen	15(3%)	93.7
e.	Plueral Effusion	9(1.8%)	177.5
f.	Pulmonary Tb AFB+	40(8%)	163.9
g.	Pulmonary Tb X-Ray +	74(14.8)	163.4
h.	Cholecystitis	1(0.2%)	64
i.	Cirrhosis Of Liver	1(0.2%)	273
j.	Bronchectasis	1(0.2%)	437
k.	Chanchroid	1(0.2%)	94
l.	Syphilis	2(0.4%)	222
m.	Gonorrhea	1(0.2%)	158
n.	Pyogenic Papular Rashes	14(2.8%)	196
o.	Pyrexia Of Unknown Origin	105(21%)	208
2.	**Viral Infections**	**85(17%)**	
a..	Herpes Zoster	72(14.4%)	206.4
b.	Hepatitis B	3(0.6%)	206.7
c.	Epstein Barr Virus	1(0.2%)	56
d.	Cytomegalo Virus	1(0.2%)	230
e.	Herpes Simples Labialis	5(1%)	308.6
F	Herpes Genitalis	1(0.2)	126
g.	Molluscum Contagiosum	2(0.4%0	338
3.	**Fungal infections**	**65(13%)**	
a.	Oral Candidiasis	46(9.2.%)	156
b.	Pnuemocystitis Zeroveci	4(0.8%)	240.9
c.	Vaginal Candidiasis	5(1%)	258.5
d.	Oesophageal Candidiasis	5(1%)	144.9
e.	Cryptococcal Meningitis	2(0.4%)	55
f.	Otomycosis	3(0.6%0	170
4.	**Parasitic Diseases**	**42(8.4%)**	
a.	Malaria	1(0.2%)	155
b.	Chronic Diarroea	40(8.0)	138.5
c.	Toxoplasmosis	1(0.2%)	296

DISCUSSION

In the present study the clinical profile of various Bacterial, Viral, Fungal and Parasitic opportunistic infections among HIV seropositive patients admitted in the Hospital were analyzed.

Table-7: Age group prevalence in HIV seropositive patients.

Authors	Age group (%)
A. Singh *et al.* 2003 [14]	92%
A. Wadhwa *et al.* 2007[15]	82%
Saldanha *et al.* 2008[16]	74.8%
Present study 2018	43.8%

Maximum numbers of HIV positive individuals (37.8%) were in the age group of 31-40 years. Several study groups both in India and abroad have reported 48.2% to 92% HIV seropositive individuals in this age group.

Male: female ratio in the present study was 1.2:1.while Saldanha *et al.* [16] reported 3:1, A.Wadhwa *et al.* [15] reported 4.8:1. Our study correlates with Saldhanha *et al.* [16] studies.

While the males belonged to a wide age spectrum, the females were a considerably younger population, and most of them acquired infection from their spouses, reflecting the male dominance in Indian society and emphasizing an increased need for awareness and counseling of both spouse.

Table-8: CD4 Count <200 mm^3 compared with other studies.

Authors	CD4 count (%)
Sharma *et al.* 2004 [17]	82.5%
A. Wadhwa *et al.* 2007[15]	60%
Anantha A. *et al.* 2012 [18]	46.2%
present study 2018	64%

The lower CD4 counts in present study may be due to a diagnostic bias from later detection of the disease reflecting a paucity of extensive diagnostic facilities at the peripheral health care centers, so that the diagnosis remains uncertain or is not established until late stages, when significant immunosuppression has already set in and patients are referred to tertiary health care centers.

The findings of low CD4 counts at admission to the hospital demonstrate that a high level of immunodeficiency was already present, defining advanced AIDS.

Table-9: Comparison of Bacterial isolates in other studies

Authors	Pulmonary TB	Extra pulmonary TB
VK Arora *et al.* 1995[19]	-	14.2%
Misra SN *et al.* 1998 [20]	66%	-
Myong *et al.* 1999 [22]	25%	-
Singh A *et al.* 2003 [14]	56%	-
Sharma *et al.* 2004 [17]	71%	-
Dungal BA *et al.* 2008 [21]	21.4%	8.92%
Ananth *et al.* 2012 [18]	52.3%	-
Pradip *et al.* 2013 [23]	17.1%	-
Present study 2018	22.8%	11.8%

.

As in the present study, pulmonary tuberculosis is the most common opportunistic infection in HIV seropositive patients. This may be due to endemicity of the causative agent. However determining the spectrum of OIs and the changing pattern over the years, in a given region requires adequate surveillance and good diagnostic services that are not available in many parts of the developing countries like India. Epidemiological features depend upon social and cultural practices of the people which may again vary from region to region.

Our observations are consistent with Dungal BA *et al.* [21] and Myong *et al.* [22] studies.

Table-10: Comparison of Viral isolates in ot her studies

Authors	Herpes simplex	Herpes zoster	CMV	EBV	Molluscum contagiosum
Myong *et al.* 1999 [22]	20%	-	-	-	-
Shobani *et al.* 2007[24]	5%	6%	-	-	-
Present study 2018	1.2%	14.4%	0.2%	0.2%	0.4%

Herpes zoster was the commonest lesion with CD4 count >400mm^3 also.

Our study correlates with Shobani *et al* [24] with CD4 count <200 mm^3.

Table-11: Comparison of Fungal isolates in other studies

Authors	Candida	Cryptococcus	Aspergillus	Pencilium	Pneumocystic jeroveci
Myoung *et al* 1999 [22]	21%	-	-	-	10%
Sharma *et al*. 2004[17]	39.3%	3.7%	-	-	7.4%
Shobhani *et al*. 2007 [24]	-	12.6%	3%	-	-
Saldnaha *et al*. 2008 [16]	34.5%	-	-	-	-
Dungal *et al*. 2008 [21]	8.9%	-	-	-	-
Ananth *et al*. 2012 [18]	39%	-	-	-	14.2%
Pradip *et al*. 2013[23]	22%	-	-	-	-
Present study 2018	11.2%	0.4%	0.3%	0.3%	-

In case of candidial infection, our study findings are comparable with findings of other studies, comparatively more incidence of candidiasis was observed in this study. Oral candidiasis was the commonest mucocutaneous opportunistic infection observed in our study. The number of T-helper cell usually fall over the course of HIV infection. Serious fungal infections tend to occur, when T-helper cell count has dropped to around 100 mm^3.

Cryptococcal meningitis is the most common type of meningitis reported in important neurological studies in India. Cryptococcal meningitis, an AIDS – defining illness, usually appears when CD4 counts are below 100/mm^3 and is associated with an increased risk of death.

Four of the HIV seropositive patients were co-infected with pneumocystis carinii pneumonia (PCP) in the present study. It is now established that PCP is one of the common opportunistic infections in HIV but the cases are relatively less documented, may be due to the lack of routine testing facility. PCP is rarely documented in India. Our results are in concordance with Dungal *et al* [21] and Myoung *et al* [22] studies with CD4 count <100.

Table-12: Comparison of Parasitic isolates in other studies

Authors	Cryptosporidium	Toxoplasmosis	Malaria
Misra SN *et al*. 1998 [20]	76%		
Singh A *et al*. 2003 [14]	47%		
Sharma SK 2004 [17]	-	3.7%	
Ananth *et al*. 2012 [18]	30%		
Pradip *et al*. 2013 [23]	14.2%		
Present study 2018	8%	0.2%	0.2%

High incidence of cryptosporidial diarrhoea was present in our study and compared with other studies with CD4 count <100 mm^3. Relatively high incidence of oral candidiasis and diarrhoea may be due to low socioeconomic status, poor hygiene and non-availability of safe drinking water. Our study correlates with Pradeep *et al*. [23] with CD4 count <100 mm^3.

Four percent of the HIV seropositive patient had polymicrobial infections, which included oral candidiasis plus pulmonary tuberculosis in 2% and PCP plus cryptosporidial infestation in 2%.

About one percent of HIV seropositive cases of present study were co-infected with Hepatitis B virus. All the co-infected patients were under gone blood transfusion previously.

CONCLUSION

HIV/AIDS is the burning crisis worldwide. The present study serves as a matrix for future evaluation. Clinicians should carefully investigate those patients with very low CD4 counts presenting with symptoms. Early diagnosis of opportunistic infections and prompt treatment improves the quality of life, increase sing the life expectancy of the patients.

REFERENCES

1. RE, S. T. C. (2015). General Clinical Manifestations of HIV Infection (Including the Acute Retroviral Syndrome and Oral, Cutaneous, Renal, Ocular, Metabolic, and Cardiac Diseases) in Bennett JE Dolin R Blaser MJ Mandell's Principles and Practice of Infectious Diseases..
2. Fauci, A.S, Chiffordlane, H. (2008)Human immunodeficiency virus disease, AIDS and related disorders. In : Lango DL, Kasper DL, Jameson JL, Fauci AS, Hauser SL, Loscalzo J, editors, Harrison's principles of internal medicine, 17th ed, Vol. I, New York : McGraw Hill, 1137-1203.
3. Ghate, M., Deshpande, S., Tripathy, S., Nene, M., Gedam, P., Godbole, S., ... & Mehendale, S. (2009). Incidence of common opportunistic infections in HIV-infected individuals in Pune, India: analysis by stages of immunosuppression represented by CD4 counts. *International journal of infectious diseases*, *13*(1), e1-e8.

4. Holmes, C. B., Wood, R., Badri, M., Zilber, S., Wang, B., Maartens, G., ... & Losina, E. (2006). CD4 decline and incidence of opportunistic infections in Cape Town, South Africa: implications for prophylaxis and treatment. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, *42*(4), 464-469.
5. Brambilla, A. M., Castagna, A., Nocita, B., Hasson, H., Boeri, E., Veglia, F., & Lazzarin, A. (2001). Relation between CD4 cell counts and HIV RNA levels at onset of opportunistic infections. *Journal of acquired immune deficiency syndromes (1999)*, *27*(1), 44-48.
6. Pavie, J., Menotti, J., Porcher, R., Donay, J. L., Gallien, S., Sarfati, C., ... & Molina, J. M. (2012). Prevalence of opportunistic intestinal parasitic infections among HIV-infected patients with low CD4 cells counts in France in the combination antiretroviral therapy era. *International Journal of Infectious Diseases*, *16*(9), e677-e679.
7. Back-Brito, G. N., El Ackhar, V. N. R., Querido, S. M. R., dos Santos, S. S. F., Jorge, A. O. C., de Macedo Reis, A. D. S., & Koga-Ito, C. Y. (2011). Staphylococcus spp., Enterobacteriaceae and Pseudomonadaceae oral isolates from Brazilian HIV-positive patients. Correlation with CD4 cell counts and viral load. *Archives of oral biology*, *56*(10), 1041-1046.
8. Pongsai, P., Atamasirikul, K., & Sungkanuparph, S. (2010). The role of serum cryptococcal antigen screening for the early diagnosis of cryptococcosis in HIV-infected patients with different ranges of CD4 cell counts. *Journal of Infection*, *60*(6), 474-477.
9. Elliott, A. M., Mawa, P. A., Joseph, S., Namujju, P. B., Kizza, M., Nakiyingi, J. S., ... & Whitworth, J. A. (2003). Associations between helminth infection and CD4+ T cell count, viral load and cytokine responses in HIV-1-infected Ugandan adults. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *97*(1), 103-108.
10. Mocroft, A., Phillips, A. N., Gatell, J., Ledergerber, B., Fisher, M., Clumeck, N., ... & EuroSIDA study group. (2007). Normalisation of CD4 counts in patients with HIV-1 infection and maximum virological suppression who are taking combination antiretroviral therapy: an observational cohort study. *The Lancet*, *370*(9585), 407-413.
11. King, C. H., Mandle, G. L., Benet, J. L., & Dolin, R. E. (2005). Douglas and benett's principles and practice of infections disease.
12. Kuby (2007). Cell and organs of the immune system. In : Text book of Immunology. 6th ed. New York : WH Freeman and Company, 30-49.
13. Center for Disease Control (1992). Revised Classification system for HIV infection and expanded Surveillance for case definition for AIDS among adolescents and adults. Morbid. Mortal. Wkly. Rec., 41, RR-41; 1993.
14. Singh, A., Bairy, I., & Shivananda, P. G. (2003). Spectrum of opportunistic infections in AIDS cases. *Indian journal of medical sciences*, *57*(1), 16-21.
15. Wadhwa, A., Kaur, R., Agarwal, S. K., Jain, S., & Bhalla, P. (2007). AIDS-related opportunistic mycoses seen in a tertiary care hospital in North India. *Journal of Medical Microbiology*, *56*(8), 1101-1106.
16. Saldanha, D., Gupta, N., Shenoy, S., & Saralaya, V. (2008). Prevalence of opportunistic infections in AIDS patients in Mangalore, Karnataka. *Tropical doctor*, *38*(3), 172-173..
17. Sharma, S. K., Mohan, A., & Kadhiravan, T. (2005). HIV-TB co-infection: epidemiology, diagnosis & management. *Indian J Med Res*, *121*(4), 550-567.
18. Takalkar, A. A., Saiprasad, G. S., Prasad, V. G., & Madhekar, N. S. (2012). Study of opportunistic infections in HIV seropositive patients admitted to Community Care Centre (CCC), KIMS Narketpally.
19. Arora, V. K., Gowrinath, K., & Rao, R. S. (1995). Extrapulmonary involvement in HIV with special reference to tuberculosis cases. *Ind J Tub*, *42*, 27.
20. Misra, S. N., Sengupta, D., & Satpathy, S. K. (1998). AIDS in India: recent trends in opportunistic infections. *Southeast Asian Journal of Tropical Medicine & Public Health*, *29*(2), 373-376.
21. Dhungel, B. A., Dhungel, K. U., Easow, J. M., & Singh, Y. I. (2008). Opportunistic infection among HIV seropositive cases in Manipal Teaching Hospital, Pokhara, Nepal. *Kathmandu University Medical Journal*, *6*(3), 335-339.
22. Oh, M. D., Park, S. W., Kim, H. B., Kim, U. S., Kim, N. J., Choi, H. J., ... & Choe, K. (1999). Spectrum of opportunistic infections and malignancies in patients with human immunodeficiency virus infection in South Korea. *Clinical infectious diseases*, *29*(6), 1524-1528.
23. Bhaumik, P., Debnath, K., & Sinha, B. (2013). Spectrum of opportunistic infections among HIV/AIDS patients of Tripura. *Journal, Indian Acad Clin Med*, *14*, 218-21..
24. Sobhani, R., Basavaraj, A., Gupta, A., Bhave, A. S., Kadam, D. B., Sangle, S. A., ... & Morde, S. N. (2007). Mortality & clinical characteristics of hospitalized adult patients with HIV in Pune, India. *Interpretation*, *101*, 1-19.