

## “Viral Mucocutaneous Manifestations of HIV Infection with Special Reference to CD4 Count”

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### ABSTRACT

**Introduction:** Viralskin and mucocutaneous disorders are common in HIV infection and may be the earliest manifestation of the disease. The spectrum of these disorders is wide and may vary in different regions due to varying prevalence of various microbial agents. Therefore, we studied the seroprevalence of HIV infection in patients presenting with skin and mucocutaneous disorders and clinical and regional epidemiological profile of seropositive patients.

**Objectives:** To know incidence of viral opportunistic infections in HIV, with special reference to CD4 counts.

**Materials and Methods:** This is a prospective study. Incidence of clinically and laboratory confirmed cases of viral opportunistic infections in HIV patients are recorded. Blood of these patients were processed for CD4 counts, by Partech Flow cytometry to assess the immune status among them.

**Results:** 85 HIV seropositive patients with viral opportunistic infections with age group of 15-45 years, attending the outpatient and inpatient department for the period of one year from June 2013-June 2014 at Chigateri General Hospital, ART center, were included in this study and CD4 counts were done among them. Out of these, 70(88.2%) patients had Herpes zoster with CD4 counts <200mm<sup>3</sup>, 5(5.8%) patients had Herpes simplex labialis with CD4 counts <200mm<sup>3</sup>(2.3%), patients had molluscum contagiosum with CD4 counts <100mm<sup>3</sup>, 1(1.1%) patient each of Herpes genitalis, Cytomegalovirus pneumonia, Ebstein virus infection with CD4 counts <200mm<sup>3</sup>(2.3%) patients of combined infections of Herpes zoster and hepatitis B observed with CD4 count <100mm<sup>3</sup>(2.3%) patients of combined Herpes zoster and molluscum contagiosum observed with CD4 count <50mm<sup>3</sup>. Commonly observed risk group were heterosexual, blood transfusion, non agriculture group, local transport worker and housewife.

**Conclusions:** In this study attempt was made to know the incidence of viral opportunistic infections in HIV seropositive patients with low CD4 counts, in which CD4 counts can be considered as clinical score, which serve as an alarm for timing of prophylaxis and a guide for therapeutic intervention.

### 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.<sup>1,2,3,4,5</sup>

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

### CD4 T lymphocytes and HIV<sup>10, 11:</sup>

Within hours of exposure to HIV, CD4 T lymphocytes are found to be infected showing active viral replication. The infected CD4 cells release virions by budding through the cell membrane or by lysis of the infected cells. The released virus particles then infect uninfected CD4 T lymphocytes. CD4 T lymphocytes also serve as important reservoirs of HIV: a small proportion of these cells carry HIV provirus integrated in the host DNA without active virus multiplication.

During the primary HIV infection, the number of CD4 T lymphocytes in the bloodstream decreases by 20% to 40%. HIV brings about the lysis of HIV infected cells as well as bystander uninfected cells using various mechanisms such as lysis of the cells infected with HIV. Billions of CD4 T lymphocytes may be destroyed every day, eventually overwhelming the immune system's regenerative capacity.

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### **HERPES SIMPLEX VIRUS (HSV):**

Recurrent oral, labial, genital and perianal HSV infections are common in HIV infected individuals and are typical in appearance in relatively immunocompetent state.<sup>1,2,22</sup> Once significant immune suppression supervenes, lesions manifest by chronic deep ulcers involving the perianal region, genitalia and tongue. Secondary bacterial infection may also be seen. Untreated ulcerative lesions usually slowly enlarge or less often become verrucous and hyperplastic. HSV infection may occur in oropharynx and extend to the oesophagus and cause severe odynophagia. Herpes Simplex folliculitis is also observed.

### **HERPES ZOSTER:**

HIV associated primary varicella may follow a benign course or it may be complicated by fatal pulmonary involvement. Lesions may become persistent, hyperkeratotic, bullous, chronic ecthymatous with central crusting or ulceration or both, surrounded by a border of vesicles. Among Indian patients, 6-9% to 25% with HIV infection have been reported to develop H.zoster during the course of the disease. It is described as an indicator of HIV infection in patients at risk. HIV infected patients have a 7-15 times greater relative risk of developing Herpes Zoster, a disease that is predictive of progression to severe immune suppression, especially if associated with fever. Most cases of Herpes Zoster develop in patients with CD4 count between 200-500 cells/mm<sup>3</sup>. HIV associated zoster may be classic dermatomal eruption, multidermatomal, ulcerative, chronic, verrucous, bullous, haemorrhagic or necrotic. Bacterial superinfection, acyclovir resistance, therapeutic failure, and multiple recurrences are not uncommon. Vasculitis with bone necrosis and exfoliation of teeth may develop.

### **CYTOMEGALOVIRUS:**

Cytomegalovirus (CMV) is the most common cause of serious opportunistic infection in patients with AIDS.<sup>19,21</sup>

However cutaneous involvement is rare and no specific CMV induced skin lesions have been identified on HIV infected individuals. Cutaneous manifestations include perianal ulcerations, keratotic verrucous lesions and palpable purpuric plaques.

### **POX VIRUS: (Molluscumcontagiosum)**

Molluscumcontagiosum (MC) appears in 3 to 10% of the cases of Indian HIV infected patients. HIV infected patients may develop

classic dome shaped umbellicated papules as well as larger (>1cm called giant MC) coalescent, extensive and persistent lesions that are often resistant to treatment. Although any part of the body can be affected, the lesions favour the face and intertriginous areas.<sup>1, 2, 21</sup>

Clinical differential diagnosis includes basal cell carcinoma and cutaneous lesions of cryptococcus and dimorphic fungi. Spontaneous regression may occur with ART. Diagnosis confirmation is by biopsy and culture.<sup>19</sup>

## **MATERIAL 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 Hospitals attached to JJM Medical College Davangere, during the one year period, from June 2013 to June 2014 form the study group.

### **Sample size:**

85 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 Herpes zoster and H.simplexlabialis, Herpes genitalis, molluscumcontagiosum.

#### **Exclusion Criteria:**

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

## **METHODS OF SPECIMEN COLLECTION:**

### **Specimen for CD4 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 PartecGmbH. Am Flugplatz 13. D-02828 Gornitz. Germany).

**CD4 Easy count (PARTECT)**

Product Name: CD4 easy count kit

Code No.: 05-8401

Content: Packing contains reagents for 100 tests  
 \* 100 ml no lyse buffer  
 \* 2000 µl cD4 mAb PE (MEM-241, PE-conjugated Monoclonal antibody to human CD4)

- Powder-free latex gloves (e.g. Safeskin, Code No. 545-950-06)
- Venous Blood Collection System with EDTA as anticoagulant (e.g. Greiner Bio-One: Vacuette® EDTA Tubes, K3E/EDTA K3, 3 ml, Code No. 454217, Vacuette® Blood Collection Needles 38 x 0.8 mm, Code No. 450076, Vacuette® Tube Holders, Code No. 450201)

**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 subpopulation 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 84ul 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.

**Recommended and Required Materials:**

- Partec Flow Cytometry instrument (e.g. CyFlow® Counter Code No. CY-S-3022 or CyFlow® SL\_3 Code No. CY-S-1023)
- Partec test tubes (Code No. 04-2000)
- Micropipettes and pipette tips (e.g. Eppendorf, Code No. 3112 000.029 and 3111 000.0165)

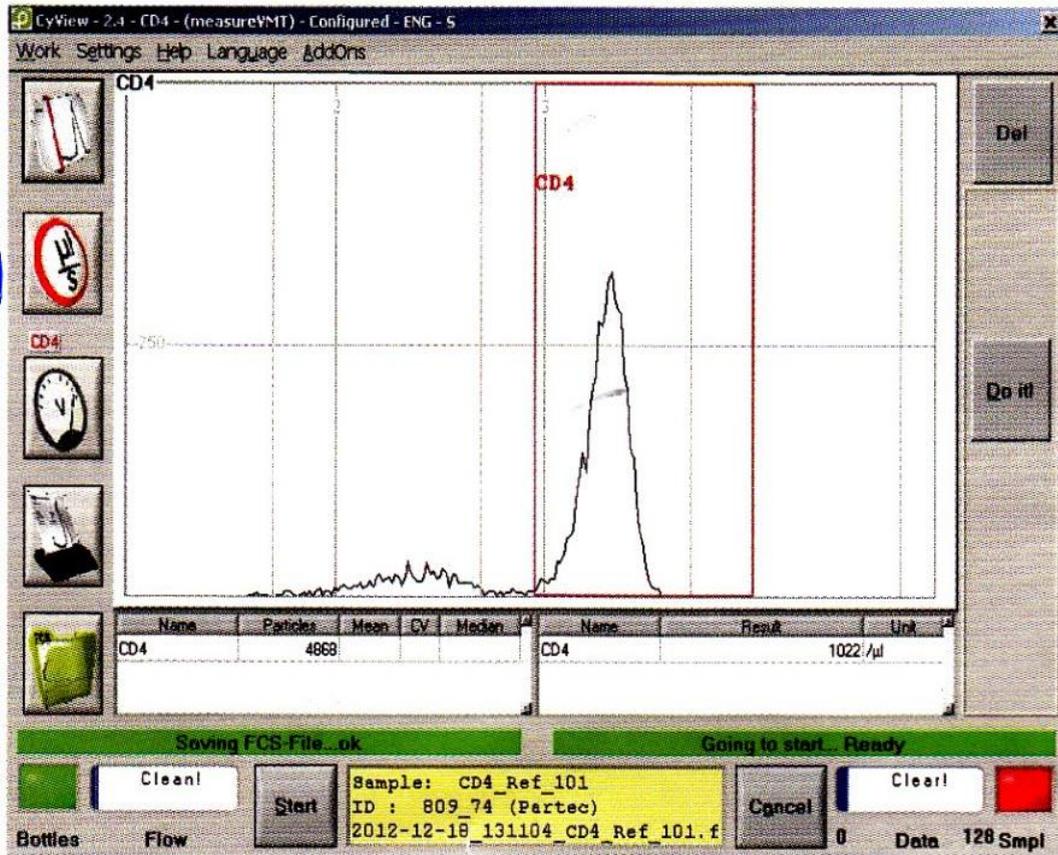
**Handling and Storage:** CD4 mAb PE is supplied in 2 ml of phosphate-buffered saline (PBS) pH 7.4, containing BSA and 0.09% sodium azide, sufficient for 100 tests. 20 pi of CD4 mAb PE are sufficient for labelling 1x10<sup>6</sup> cells. When stored at 2-8°C in the dark, the CD4 easy count kit is stable until the expiration date printed on the kit label. Do not freeze or expose to elevated temperatures.

**Method:**

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

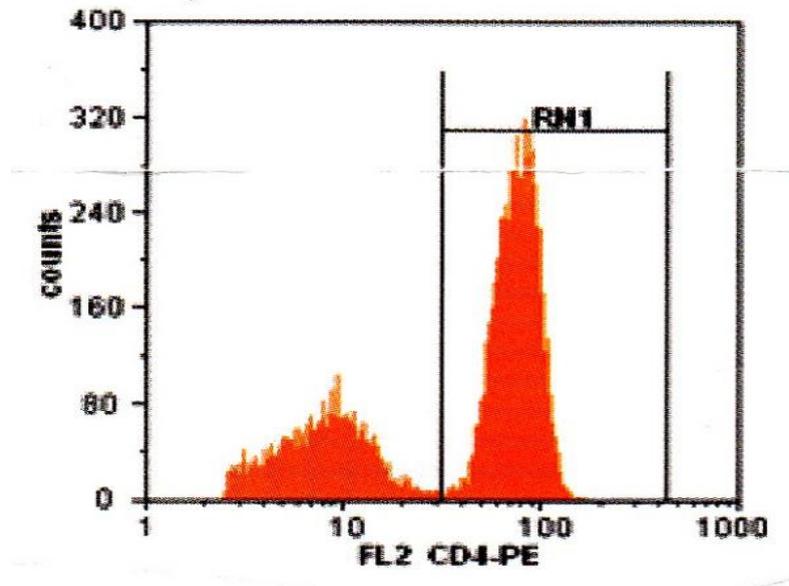
**Direct counting result using the CD4 easy count kit:**

Screenshot from a PartecCyFlow® counter. As depicted in the histogram, the CD4<sup>+</sup> T-cells (CD4 gage, prominent peak on the right) can be clearly separated from the CD4<sup>+</sup> monocytes (left peak of weaker fluorescence intensity). The absolute concentration of CD4<sup>+</sup> T-cells is displayed directly as number of cells/µl whole blood.



**Direct CD4 counting result using CD4 easy count kit:**

Display of a PartecCyFlow® SL\_3. The FL2 CD4-PE histogram shows the precise separation of the CD4<sup>+</sup> T-cells (cell population in the RN1 range) from the CD4<sup>+</sup> weak monocytes.





**Comb Aids, Triline and Tridot reagents with tests**



**Sample processing Vacutainer for CD4 count analysis**



**CD4 counts Reagents kits**  
(No lyse buffer, conjugated monoclonal antibody to Human CD4)



**Automated Flow Cytometry analyzer - PartecCyflow counter®**

**RESULTS**

The present study was carried out on 85 HIV seropositive patients with signs and symptoms of opportunistic infections attending Chigateri District Hospital, ART center, J.J.M. Medical College, Davangere, over a period of 12 months (June 2013 to May 2014), to know the incidence of bacterial, viral and fungal, parasitic infections and their correlation with CD4 count.

The observations made from the study are shown in the following tables, present study includes age group of <20yrs to >70 yrs with male preponderance with male: female ratio was 1.2:1. Commonly observed risk group were heterosexual, blood transfusion, non agriculture group, local transport worker and house wife.

**Table 1: Showing Viral OIS with Mean CD4 Counts**

Herpes Zoster	72	84.7	206.4
Hepatitis B	3	3.5	206.7
Epstein Barr Virus	1	1.1	56
Cytomegalo Virus	1	1.1	230
Herpes Simples Labialis	5	5.88	308.6
Herpes Genitalis	1	1.1	126
MolluscumContagiosum	2	2.3	338

Out of 85 cases, 72(84.7) cases of Herpes zoster had mean CD4 count of 206.4 mm<sup>3</sup>. 3(3.5%) cases of hepatitis rashes had CD4 count of 7mm<sup>3</sup>. 206.5(5.88%) Herpes labialis had CD4 count of 308.6 mm<sup>3</sup>. 2 cases molluscumcontagiosum had CD4 count of 338 mm<sup>3</sup>. (1.1%) each case of Herpes genitalis and CMV infections with CD4 count of 126mm<sup>3</sup> and 338mm<sup>3</sup> respectively.

**Table 2: Showing Viral OIS with CD4 Counts**

CD 4 count	Herpes zoster	Hepatitis B	Epstein Barr virus	Cytomegalo virus	Herpes simplex labialis	Herpes genitalis	Molluscum contagiosum	Total
<100	13	2	0	0	1	0	1	17
101-200	22	0	1	0	3	1	1	28
201-300	19	1	0	0	0	0	0	20
301-400	13	0	0	1	1	0	0	15
401-500	1	0	0	0	0	0	0	1
501-600	3	0	0	0	0	0	0	3
601-700	0	0	0	0	0	0	0	0
>701	1	0	0	0	0	0	0	1
<b>Total</b>	<b>72</b>	<b>3</b>	<b>1</b>	<b>1</b>	<b>5</b>	<b>1</b>	<b>2</b>	<b>85</b>

85 (17%) cases were viral infections. Among 85 cases, 72 (84.7%) cases were Herpes zoster, 5 (5.88%) cases were Herpes labialis, 3(3.5) cases were Hepatitis B, 2(2.3%) cases were molluscumcotagiosum. Each 1(1.1) case had CMV and EBV respectively with CD4 count <100mm<sup>3</sup>.

17(20%) cases had CD4 count <100 mm<sup>3</sup>, indicated by major viral lesions like Herpes zoster, Hepatitis B, Herpes simplex labialis and Molluscum contagiosum. 28(32.9%) cases had CD4 count 101-200mm<sup>3</sup>. 20(23.5%) cases had 201-300mm<sup>3</sup>. 15(17.6) cases had 301-400mm<sup>3</sup>. 3 cases had 501-600. 1(1.1) each case had CD4 count 401-500 and >700 respectively.



**Disseminated Herpes Zoster**



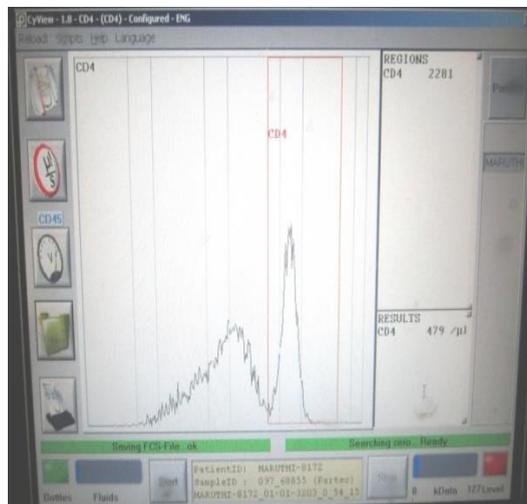
**Herpes zoster**



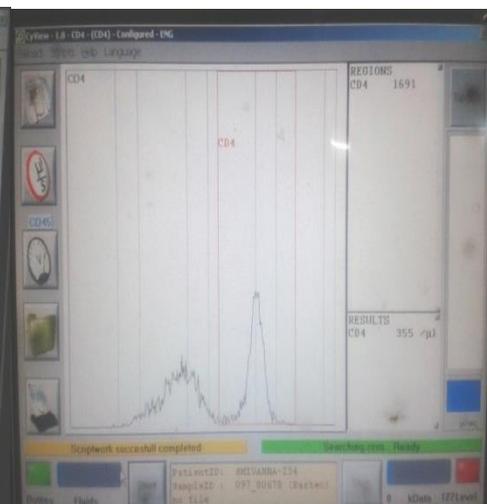
**Herpetic stomatitis**



**Extensive mollusumcontagiosum**



**Graph of CD4 count (>400)**



**Graph of CD4 count (>300)**

**DISCUSSION**

Opportunistic infections occur with remarkable frequency and cause substantial morbidity and mortality among patients with AIDS. The HIV epidemic in India is geographically diverse and there are regional differences, not only in the incidence and prevalence of HIV infections, but also in the burden of background communicable diseases. Identification of specific pathogen is very important for management of such cases.

In the present study the clinical profile of various Bacterial, Viral, Fungal and Parasitic opportunistic infections among HIV seropositive patients admitted in the Chigateri District Hospital and Bapuji Hospital attached to J.J.M. Medical College, Davangere were analyzed.

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. Vickers et al reported 1.4:1, while Saldanha et al reported 3:1, Ghate et al reported 4:1, A.Wadhwa et al reported 4.8:1, Rosy Parmar et al reported 3.5:1, Pradeep et al reported 2.7:1. Our study correlates with Pradeep et al. 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.

**CD4 Count <200 mm<sup>3</sup> compared with other studies**

Authors	CD4 count (%)
Sharma et al <sup>124</sup> 2004	82.5%
A. Wadhwa et al <sup>99</sup> 2012	60%
Anantha A. et al <sup>93</sup> 2012	46.2%
Ghate et al <sup>88</sup> 2009	69.6%
Michael O. Iroezindu et al <sup>95</sup> 2013	95%
present study 2014	64%

Progression and HIV infection is largely dependent on the interaction between the viral and host factors. HIV brings about the destruction of CD4 lymphocytes which are the crucial cells in forming immune response to foreign antigens and it is also primary target cells of HIV.<sup>12</sup>

The progressive loss of these lymphocytes even today results in the loss of an ability to mount desirable immune response to any pathogen and death of patients in terminal stage and HIV infection occurs. Major cause of morbidity and mortality of such patients are opportunistic pathogens.<sup>11,13</sup>

**Viral isolates:**

Authors	Herpes simplex	Herpes zoster	CMV	EBV	Molluscum contagiosum
Myong et al <sup>133</sup> 1999	20%	-	-	-	-
Ghate et al <sup>88</sup> 2009	10%	-	-	-	-
Nilanjan Chakravarty <sup>107</sup> , 2000	-	-	45%	-	-
Sepkowitz et al <sup>49</sup> 2002	-	-	2%	-	-
PV Krishnarao <sup>30</sup> 2005	7%	16%	-	-	12%
Shobana et al <sup>51</sup> 2004	5%	6%	-	-	-
Balkhair et al <sup>131</sup> 2012	-	-	8%	-	-
Present study	1.2%	14.4%	0.2%	0.2%	0.4%

Our study correlates with Shobana et al, and PV Krishnarao et al with CD4 count <200 mm<sup>3</sup>.

## CONCLUSION

- HIV/AIDS is the burning crisis worldwide.
- Un AID aims to deliver prevention activities while in high prevalence countries it is implementing a programs that includes care and support activities with control of AIDS and STDs.
- Early diagnosis of opportunistic infections and prompt treatment improves the quality of life, increases the life expectancy among infected patients and delays progression to AIDS.
- Clinicians should carefully investigate those patients with very low CD4 counts presenting with symptoms suggestive of meningitis. Early diagnosis and effective treatment may considerably reduce the morbidity and mortality associated with this condition.
- The type of pathogens responsible for opportunistic infections varies from region to region.
- Identification of a specific pathogens is very important for the management and such cases.
- Majority of opportunistic infections, were reported in the age group of 31-40 years (37.8%) with male (55.2%) majorities group with ratio of 1.2:1. There is a male preponderance over female, with maximum patients from sexually active age group. Hence one should focus on this age group especially male group for the prevention of high rate of HIV transmission.
- Our study group compromised of predominate high risk group was heterosexual group (86.8%) and occupational group was agricultural labourer (31.4%), predominant WHO grading was III (55.4%). There was statistical significant association between CD4 count and age risk group WHO grading.
- Among viral infections (17%) predominant infection was Herpes zoster (14%) with mean CD4 count <200.
- Most of the patients (32.4%) had CD4 counts <100 mm<sup>3</sup> indicating advanced stage of the stage, followed by <200 mm<sup>3</sup> (31.2%).
- The study provides importance information about the risk of commonly reported OIs at lower CD4 counts. These results highlight the need for early screening a also the need to increase awareness in health care providers in order to improve decisions regarding prophylaxis for prevention and opportunistic therapeutic interventions.
- Our study will also help program manages to plan appropriate strategies for the investigation a treatment of common OIs as a part of management package for HIV infected populations.
- Timely initiation and continuous intake of ART will not only prolong the survival but will also decrease the viral load a transmission of the disease.
- With better knowledge and diagnosis of the opportunistic infections in HIV patients, clinicians and health planners can tackle the AIDS epidemic in a more effective manner.
- Specific antimicrobial prophylaxis by itself or in conjunction with antiretroviral therapy can reduce the substantial morbidity and mortality caused by opportunistic infections in patients with HIV infection.
- Early diagnosis of opportunistic infections and prompt treatment definitely contribute to increased life expectancy among infected patients delaying the progression to AIDS.
- This study once again proves that the spectrum of opportunistic infections among various patient groups varies significantly. This study is the first ever reported data on OIs among HIV/AIDS patients from Davangere. This will serve as a matrix for future evaluation.
- The pattern of opportunistic infections in a particular area helps the attending physicians to be on the look out for them and take prompt therapeutic measures.
- Simultaneously specific health education of PLWHA regarding early detection of opportunistic infection (OI) and importance of antimicrobial prophylaxis to reduce the morbidity and mortality can be undertaken.
- The positive outcome can be attributed to familistic orientation of Indian society and spread of awareness about HIV/AIDS which can be managed by mutual understandings amongst couple.

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