

### **Study of clinical manifestations of HIV infected children in relation to CD4 count**

#### **ABSTRACT**

**Aims:** To study the clinical profile of HIV infected children and correlate them with the CD4 count at the time of presentation.

**Study design:** Hospital based observational study

**Place and duration of study:** ART centre of SCBMCH and Department of Pediatrics, SVPPGIP, Cuttack, Odisha during the period October 2017 to September 2019.

**Methodology:** All children aged between 18months to 15 years who were confirmed to be HIV positive by ELISA or immunocomb II, either at present or past were included in the study. They were subjected to CD4 count testing, along with nutritional assessment and screened for opportunistic infections, apart from their socio-demographic details. All quantitative variables including age, weight, height, were compared by unpaired t-test. Categorical variables like sex mode of transmission were compared by chi-square test. Variables like clinical staging, immunological staging and CD4 counts were measured at presentation and analyzed with repeated ANOVA at 5% level of significance to allow for multiple comparisons.  $P < 0.05$  was considered was significant and inferences were drawn.

**Results:** Out of 103 cases, majority belonged to age 4- 7 years, and 59.2% were malnourished. 24.3% were asymptomatic, with the most common clinical features noted were fever (65%), cough (47.8%), recurrent diarrhoea (41.8%) and weight loss (40.8%). The incidence of opportunistic infections was 24.3%, with tuberculosis (40%) and herpes zoster (36%) leading the list. Most cases (37.87%) came under WHO clinical stage-III. A significant correlation was found between CD4 count and age, WHO clinical staging, opportunistic infections in the population. Lower CD4 counts were associated with younger age, lower staging and less risk for symptoms and infections.

**Conclusion:** CD4 count is a reliable marker to assess the staging and risk for opportunistic infections in pediatric HIV and thus can be used as screening tool for complications/ deterioration in the child, for better management.

**Keywords:** HIV, CD4 count, clinical profile, opportunistic infections

## 1.INTRODUCTION

Human immunodeficiency virus (HIV) infection has established itself as a leading cause of childhood morbidity and mortality in India. With nearly 2lakh of the Indian pediatric population being affected, pediatric HIV in India contributes the second highest load in the world.<sup>[1,2]</sup>

50% of the infected children under 15 years die within 2 years constituting about 18% of 3.3 million AIDS death every year.<sup>[3]</sup> The remaining infected 50% children have a slower rate of disease progression, but still suffer significant morbidity in the form of growth failure, opportunistic infections and psychological disturbances.

Children with HIV have a higher viral load, weaker immune system, variable latency period, different spectrum of clinical manifestations and patterns of disease progression, which makes them different from adults, especially in terms of treatment and monitoring. However, like adults, children with HIV also develop life threatening opportunistic infections like toxoplasmosis, tuberculosis, pneumocystis carinii pneumonia, lymphocytic interstitial pneumonitis, severe candida infection, chronic diarrhoea, lymphadenopathy, fever, CMV infection<sup>[2]</sup>

With increased availability of the equipment to test CD4 count and the knowledge that CD4 cells are the primary target of HIV, CD4 count estimation and CD4% have been considered the most reliable markers of disease progression and immune status in HIV infected patients.<sup>[4,5]</sup> Children with opportunistic infection have lower CD4 values compared to others without opportunistic infections. Tuberculosis is the commonest opportunistic infection with low CD4 ratio ranging from 6-32%. HIV encephalopathy which related to poor outcome for survival having CD4 ratio of 7%. Further, there seems to be a proportionate deterioration of WHO clinical stage of disease with decrease in CD4%.<sup>[6,7]</sup>

Owing to the ready availability of CD4 facilities in ART centres, and ease of testing compared to other extensive tests to determine immunodeficiency, CD4 count can be incorporated into the treatment protocol, and establish guidelines for initiation of treatment and prophylaxis of various clinical conditions including opportunistic infections in HIV infected children. By knowing the clinical spectrum of HIV infection in our region, screening can be intensified on the suspected children and early diagnosis can be made, which will help in early management and in decreasing the incidence of HIV related morbidity. This study is needed to thus identify the early clinical features of HIV infection and correlate with the CD4 count.

## **2.MATERIALS AND METHODS:**

**2.1. Study place:** ART centre of SCBMCH and Department of Pediatrics, SVPPGIP, Cuttack, Odisha

**2.2 Study period:** From October 2017 to September 2019

**2.3 Study design :** A Hospital based observational study

### **2.4 Inclusion criteria:**

1. All confirmed HIV positive children diagnosed by ELISA and immunocomb II.

### **2.4 Exclusion criteria:**

1. All HIV positive children, whose age more than 14 years and below 18 months
2. All HIV positive children within 18 months to 14 year whose parents are unwilling to give consent.

Screening for HIV was done after a written informed consent from the parents or care givers. Diagnosis of HIV was confirmed by ELISA using two different antigens and rapid tests. Children <18 months were excluded from the study as their diagnosis done by PCR.

### **2.5 Methodology:**

After obtaining informed consent from the parents/ care-givers of the child, the clinical and demographic information were noted, including possible mode of transmission, history and clinical findings, nutritional and developmental status, staging of disease and presence of opportunistic infections, using a pre-structured proforma. Special investigations were performed if clinically indicated (depending on the symptomatology at presentation).

Nutritional status of the children was assessed by anthropometry. Anthropometric measurements included height, weight and skin fold thickness. As per WHO Global database on Child Growth and Malnutrition recommendations, a cut off z score of <-2 was used to classify low weight-for-age (underweight), low height-for-age (stunting) and low weight-for-height (wasting) as moderate and z score <-3 to define severe malnutrition.

Opportunistic infections were diagnosed using Standard protocol. Children were categorized by their presenting complaints into clinical staging (I to IV) as per WHO clinical classification for HIV. Baseline value of Hemogram, Liver function test, renal function test was obtained in all children.

Baseline CD4 lymphocyte count were determined by FACS count (Becton & Dickinson). It is done by as an absolute number derived by multiplying the FACS's percentage of CD4 cells by the total lymphocyte count. Immunological assessment was done in terms of CD4 count as per WHO classification.

**2.6 Data analysis:** All quantitative variables including age, weight, height, were compared by unpaired t-test. Categorical variables like sex mode of transmission were compared by chi-square test. Variables like clinical staging, immunological staging and

CD4 counts were measured at presentation and analyzed with repeated ANOVA at 5% level of significance to allow for multiple comparisons.  $P < 0.05$  was considered significant and inferences were drawn.

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### 3.RESULTS

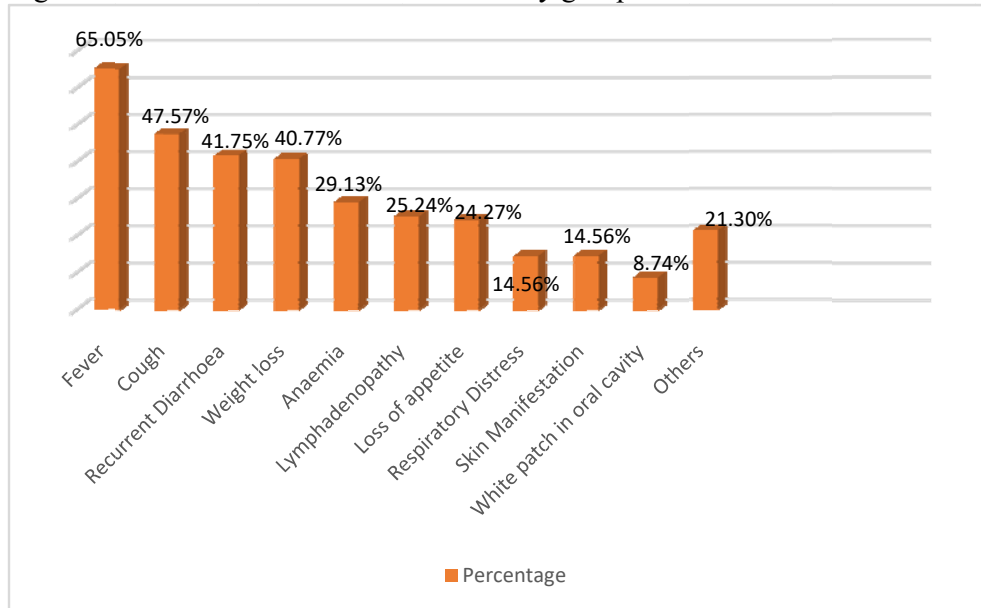
A total of 103 children infected with HIV were enrolled in the study, of which 60(58.3%) were male, with the male to female ratio being 1.4. Majority of the children (42.7%) belonged to age group between 4 to 7 years and 40.8% of the children were in the lower middle class in terms of socioeconomic status. The common mode of acquiring infection was parent to child transmission, constituting 94.2% of the population. On assessing the nutritional status of the children, it was noted that 40.8% had normal nutritional status, 24.2% were stunted and wasted, whereas 18.5% were stunted alone. Further details are mentioned in table 1, given below.

Table1. Characteristics of the study population

Variable	No. of patients	Percentage
<b>Sex</b>		
Male	60	58.25
Female	43	41.75
<b>Age group</b>		
18 mo- <4yr	26	25.2
4yr- <7yr	44	42.72
7yr- <10yr	17	16.50
10yr- <14yr	16	15.54
<b>Socioeconomic Status</b>		
Lower	22	21.36
Upper Lower	25	24.27
Lower Middle	42	40.77
Upper Middle	10	9.70
Upper	4	3.9
<b>Nutritional Status</b>		
Normal	42	40.78
Wasted	17	16.51
Stunted	19	18.46
Wasted and Stunted	25	24.25
<b>Mode of transmission</b>		
Mother to child Transmission	97	94.17
Blood Transfusion	2	1.94
Needle prick injury	1	0.97
Not Known	3	2.92

Out of 103 children studied, 25(24.3%) were asymptomatic. However, the rest of the population showed a variety of clinical features, the common ones being fever (65%), cough (47.8%), recurrent diarrhoea (41.8%) and weight loss (40.8%). The other clinical features are depicted in figure 1 below.

Figure 1: Clinical manifestations of the study group



The incidence of opportunistic infections present in the study population was 24.3%. Leading in the list was tuberculosis (40%) followed by herpes zoster (36%) and oral candidiasis (12%). [Figure 2] Among the patients with tuberculosis, 50% had pulmonary tuberculosis, 20% had disseminated tuberculosis and 30% had extra-pulmonary tuberculosis.

Figure 2. Opportunistic infections present in study group

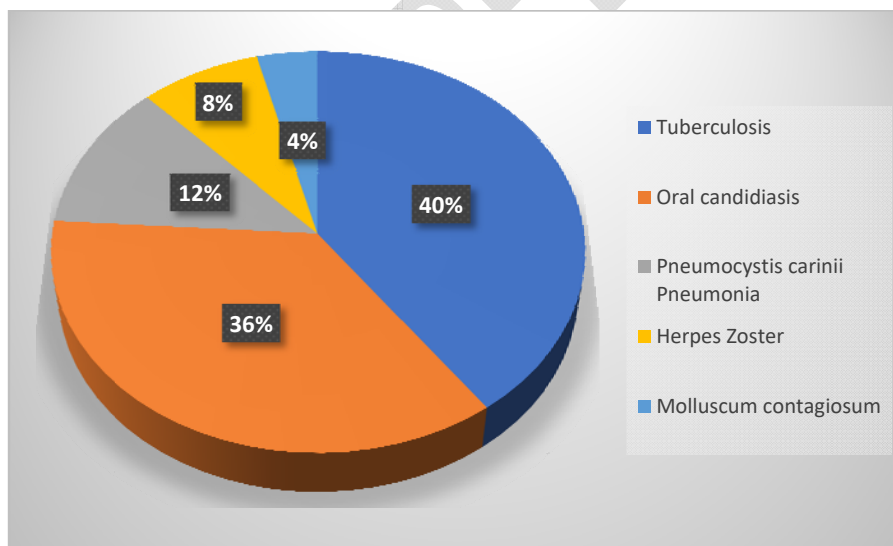


Table 2 shows categorization of children in WHO clinical staging at the time of presentation. Out of 103 cases maximum 39(37.87%) children came under clinical stage-III followed by 27(26.21%) children in clinical stage-II, 25(24%) children in stage-I and 12(11.65%) in stage-IV.

A further correlation was seen between CD4 count and WHO staging, with correlation factor being 0.66 and *P* value 0.001. This correlation showed a decreasing trend with transition from stage I to stage IV, with the mean CD4 count being 906.48 in stage I and 113.83 in stage IV.

Similarly, correlation was also established between CD4 count and specific opportunistic infections the child presented with. The children infected with HIV and suffering from herpes zoster had higher CD4 count and those with tuberculosis and pneumocystis carinii pneumonia had lower CD4 count.

With respect to age as well, CD4 count showed an inverse relation with age of the child, as seen in table 2. This association was further found to be statistically significant with *p*-value being 0.01.

Table 2. CD4 count versus the WHO staging, opportunistic infections and age of the child

WHO Clinical Staging	No. of Patients	Mean CD4 counts	<i>P</i> value
I	25	906.48+493.25	0.01
II	27	560.15_+192.67	0.01
III	39	307.97+-213.76	0.001
IV	12	113.83+_79.45	0.04
<b>Opportunistic infection</b>			
Herpes zoster	2	341.55+_33.23	0.053
Oral candidiasis	9	204.11+_110.42	
Molluscum contagiosum	1	157	
Tuberculosis	10	141.88+_115.29	
Pneumocystis carinii pneumonia	3	95.66+_33.33	
<b>Age group</b>			
18 mo-4 year	26	778.56+_649.21	0.01
4-7 year	44	552.09+_456.76	
7-10 year	17	408.82+_391	
10-14 year	16	404.43+_308.32	

Further, on comparing the CD4 counts of those with opportunistic infection and those without, a significant difference was seen (*P* value 0.01). The mean CD4 count of those with infection was 174.32, while those without opportunistic infection had higher count (574.43).

In fact, the mean CD4 value of asymptomatic patients (906.48\_+493.25), was higher than that of symptomatic cases (327.32\_+255.12). It showed asymptomatic had higher immune status than those present with symptoms which is statistically significant (*P* value 0.001).

#### 4.DISCUSSION

Ever since its discovery in 1981, HIV has rapidly spread to all parts of the world, causing it to be declared a pandemic.<sup>[8]</sup> Despite all the recent updates and innovations in treatment and management strategies, the burden of HIV is long and extensive, be it in terms of mortality, morbidity, financial constraints, reduced quality of life or the associated stigma. Thus, parallel to the attempts in discovering a final cure, measures for prevention, early detection and improvement of outcome have to be highlighted. Owing to the high mortality associated with opportunistic infections in HIV children, the clinical features and correlation with CD4 count has been revisited in this study.

In the present study, 103 children who were diagnosed with HIV were enrolled. Males outnumbered females, with the male to female ratio being 1.4:1. Majority of the children were in age group 4 to 7 years, similar to study done by Ramaswamy et al<sup>[2]</sup> and Sunder et al<sup>[9]</sup>. The maximum no of cases in our study were from 4-7-year age which might due to the lack of early detection due to non-availability of proper laboratory facilities, or not coming for prolonged follow up or death of children due to opportunistic infection before reaching higher age group.

In terms of nutritional status, though 40.8% did not have malnutrition, 16.5% and 18.5% were wasted and stunted respectively, in our study population. 24.2% of our population had both stunting and wasting. These results are comparable to those reported by Agarwal et al.<sup>[6]</sup> and Krishna et al<sup>[10]</sup> where the rates of malnutrition were around 56%. Furthermore, the commonest mode of acquisition of HIV in these children was parent to child transmission (94.2%), which was on the same lines as studies done by Ramaswamy et al<sup>[2]</sup> and Lodha et al<sup>[11]</sup>.

The clinical manifestations in the study group were varied. Out of 103, 24.3% were asymptomatic. Among those who had symptoms, fever was observed in 65% of the population, followed by cough (47.57%), recurrent diarrhea (41.75%) and weight loss (40.77%). Hematological manifestations such as anaemia (29.13%), lymphadenopathy (25.24%) and skin manifestations (14.56%) were also seen. A study by Agarwal et al<sup>[6]</sup> showed fever was the most common presenting symptom (53%), followed by chronic diarrhea (36%), cough (29%), generalized lymphadenopathy (24%), hepatosplenomegaly (12%) and skin manifestations (12%). Other study by Gomber et al<sup>[12]</sup> showed the similar results.

In our study population, 25(24.3%) children were presented with opportunistic infections. Tuberculosis 10(40%) was the most common opportunistic infection noted. Studies done by Agarwal et al.<sup>[6]</sup> and Lodha et al.<sup>[11]</sup> also made a similar observation about tuberculosis being the most prevalent opportunistic infection in these children. This may be due to high endemic area with high prevalence rate and low socio-economic status and malnutrition, which render these HIV infected children more susceptible to tuberculosis. Among these patients with tuberculosis, 50% had pulmonary tuberculosis, 20% had disseminated tuberculosis and 30% had extra-pulmonary tuberculosis. Other opportunistic infections noted included oral candidiasis (36%) as the second most opportunistic infection followed by pneumocystis Carinii pneumonia (12%), herpes zoster (8%) and molluscum contagiosum (4%).



Upon categorization of children according to WHO clinical staging at the time of presentation, out of 103 cases most children (37.86%) were placed in stage III followed by 26.21% in stage II and 11.65% children in stage IV. On a similar note, the study by Agarwal et al<sup>[6]</sup> showed that out of 101 cases, 40.2% children in stage III, 33.3% in stage I, 21.6% children in stage II and 4.9% children in stage IV. The study done by Ramaswamy et al<sup>[2]</sup> also established a similar trend.

Since HIV primarily targets CD4 cells, CD4 count can be used as a reliable marker to assess the immune status of the patients and also draw associations with the opportunistic infections that may follow. Significant correlation was noted between CD4 count and age, WHO staging and the opportunistic infections.

In our study, cases within 18mo-4 yr have highest mean CD4 count (778.56) and between 10-14 yrs of age had lowest CD4 count (404.43). As the age increases, there is a decreasing trend in the CD4 count. The results of our study were comparable to the study by Agarwal et al<sup>[6]</sup> which showed an inverse relation between age and CD4 count, where mean CD4% was 23.4% within 18 month-4 year group and 16.7% between 10-14 year age group. Similarly, Ramaswamy et al<sup>[2]</sup> also observed a similar pattern.

The other parameter that showed a significant association was presence of symptoms. The patients who were symptomatic were lower mean CD4 count (327.32) as compared to those with symptoms (906.48). The difference between the two groups would probably be a reflection of their immune status and measure of their ability to resist the effects of the virus or other infections. Further, this association was noted to be statistically significant ( $P$  value 0.001).

On probing deeper, it was also noted that those cases with opportunistic infections, on an average had lower CD4 counts ( $P$  value =). Children with opportunistic infection had lower CD4 count of 174.32  $\pm$  112.31, while those without opportunistic infection had higher mean CD4 count of 574.43  $\pm$  411.18. Likewise, Agarwal et al<sup>[6]</sup> also drew similar conclusions in their study.

On assessing individual opportunistic infections versus the CD4 count in our study, the mean CD4 count in tuberculosis patients was 141.88  $\pm$  115.29, out of which children suffering from pulmonary TB mean CD4 count was 203 while in extra pulmonary TB mean CD4 count was 122.5 and in disseminated TB was 44.66. Among opportunistic infection children having herpes zoster infection had higher CD4 count (341.55  $\pm$  33.23) and children with disseminated TB had lower CD4 count. Similar findings were observed also in studies by Ramesh Pol R<sup>[13]</sup>. In our study although CD4 count showed decreasing trend but  $P$  value is 0.053 which depicted that data is not significant that might be due to fact that the immunity not decreased to such level to make the data significant.

The correlation between CD4 count and WHO clinical staging showed a decreasing trend with transition from stage I to stage IV. Mean CD4 count in stage I is 906.48  $\pm$  493.25, in stage II mean CD4 count is 560.15  $\pm$  192.67, in stage III 307.97  $\pm$  213.760 and in stage IV 113.83  $\pm$  79.45. Similar results were observed in a study by Ramaswamy S et al<sup>[2]</sup> where CD4 ratio was on the higher side in stage I and II diseases and it is lower for stage III and

lowest in stage IV. Another study by Agarwal et al<sup>[6]</sup> also show similar results which showed CD4 value declined with deterioration of WHO clinical staging of diseases.

Thus, it can be said that CD4 count can be safely used as a reliable marker for clinical staging and risk of opportunistic infections in pediatric HIV. The meticulous use of CD4 count should thus be inculcated at every follow up and point of contact in the management of pediatric HIV. Guidelines for infection screening, predicting the risk for particular opportunistic infection and marking the recovery or transition from one clinical stage to another, should be incorporated based on CD4 count, which is not only reliable but objective as well. Segregating the children based on the same, will identify groups at higher risk and perhaps aid in detection of these diseases well ahead in time, such that both health care workers and families can anticipate and prepare themselves for the treatment and course that is likely to follow.

## **5.CONCLUSION**

Pediatric HIV contributes a significant burden to childhood mortality and morbidity in the Indian context, which necessitates use of markers like CD4 count, in order to predict the risk and course of the disease in individual children. In a developing country like ours, where population is large but resources are limited, the use of such a tool will aid in formulation of protocols and resource allocation, such that better and higher management is done for those who require it more, and perhaps earlier than the rest.

**6.Declarations:** None

**7.Consent:** Taken from parents/ attendant

**8.Ethical clearance:** Taken from Institutional Ethical Committee.

## **9. References**

- 1) Paranjpe SM, Sarkate PP, Ingole NA, Raut SS, Mehta PR. Profiles of HIV-infected anti retro viral therapy naïve children from Mumbai, India World J Pediatr. 2016 Nov;12(4): 430-435
- 2) Ramaswamy S, Thandavarayan M, Thirumalaikuruswamy S, Sureshkumar A. A study on clinical profile of paediatric HIV infection in the age group of 18 months to 12 years and its correlation with CD4 count, Int J Contemp Pediatr. 2017;4:1232-1235
- 3) Joint United Nations Programme on HIV/AIDS (UNAIDS) and World Health Organization (WHO). AIDS epidemic update: December 2003. UNAIDS, WHO. 2003.
- 4) WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV related disease in adult and children. WHO. 2006. Available from: URL: [http://www.who.int/hiv/pub/guidelines/HIV\\_staging150307.pdf](http://www.who.int/hiv/pub/guidelines/HIV_staging150307.pdf). Accessed September 18, 2018
- 5) Gupta P, Shah D. Protein Energy Malnutrition. In: Ghai OP, Gupta P, Paul VK, editors. Ghai Essential Pediatrics. 6th ed. New Delhi: CBS Publishers & Distributors; 2004. p. 101-118.
- 6) Agarwal D, Chakravarty J, Sundar S, Gupta V, Bhatia BD. Correlation between clinical features and degree of immunosuppression in HIV infected children. Indian Pediatr. 2008 Feb;45(2):140-3

- 7) Shah I. Correlation of CD4 count, CD4% and HIV viral load with clinical manifestations of HIV infected Indian children. *Ann Trop Pediatr* 2006; 26: 115-119.
- 8) Joint United Nations program on HIV/AIDS (UNAIDS)/WHO. AIDS epidemic update. 2006. Available at URL: <http://www.unaids.org/en/Publications/default.asp>. Accessed on 1 October 2007.
- 9) Attili SVS, Singh VP, Rai M, Varma DV, Gulati AK, Sundar S. Hematological profile of HIV patients in relation to immune status- A hospital based cohort from Varanasi, North India. *Turk J Haematol* 2008;25(1):13-19.
- 10) Swetha GK, Hemalatha R, Prasad UV, Murali V, Damayanti K, Bhaskar V. Health and nutritional status of HIV infected children in Hyderabad, India. *Indian J Med Res*. 2015 Jan; 141(1): 46-54
- 11) Lodha R, Singhal T, Kabra SK. Pediatric HIV infection: clinical manifestation and diagnosis. *Ann Natl Acad Med Sci (India)*. 2000;36:75-82.
- 12) Gomber S, Kaushik JS, Chandra J and Anand R. Profile of HIV infected children from Delhi and their response to ART. *Indian Pediatr* 2011;48:703-707.
- 13) Pol RR, Shepur TA, Ratageri VH. Clinico-Laboratory Profile of pediatric HIV in Karnataka. *Indian J Pediatr*. 2007;74(12):1071-5. 33) Joint United Nations Program on HIV/AIDS (UNAIDS)/WHO AIDS epidemic update 2012

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