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CLINICAL & BASIC RESEARCH

Opportunistic Infections and Complications in Human Immunodeficiency Virus-1-Infected Children

Correlation with immune status

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العدوى الانتهازية والمضاعفات عندالإصابه بفيروس نقص المناعة البشرية–1 في الأطفال والارتباط مع الحالة المناعية

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abstract: ***Objectives:*** The aim of this study was to ascertain the correlation between various opportunistic infections and complications in human immunodeficiency virus (HIV)-1-infected children and the immune status of these patients, evaluated by absolute cluster of differentiation 4 (CD4) count and CD4 percentage. ***Methods:*** This study was conducted from January 2009 to June 2010 at the Antiretroviral Treatment Centre of the Pt. B.D. Sharma Post Graduate Institute of Medical Sciences, a tertiary care hospital in Rohtak, Haryana, in northern India. A total of 20 HIV-1-infected children aged 4–57 months were studied. Demographic and baseline investigations were performed prior to the start of highly active antiretroviral therapy (HAART). A fixed-dose combination of HAART was given based on the patient's weight. Baseline investigations were repeated after six months of HAART. ***Results:*** There was a significant increase in the patients' haemoglobin, weight, height and CD4 count after six months of HAART. Significant improvements (P <0.05) were also noted in the patients' immune status, graded according to the World Health Organization. ***Conclusion:*** This study observed that the severity and frequency of opportunistic complications in paediatric patients with HIV-1 increased with a fall in the CD4 count. The treatment of opportunistic infections, along with antiretroviral therapy, may lead to both clinical and immunological recovery as well as a decreased incidence of future opportunistic infections. The CD4 count may give treating physicians an initial idea about the immune status of each child and could also be used as a biological marker of HAART efficacy. Patient compliance must be ensured during HAART as this is a key factor in improving outcomes.

Keywords: AIDS; Highly Active Antiretroviral Therapy; AIDS-Related Opportunistic Infections; CD4 Lymphocyte Count; India.

الملخص: الهدف: الهدف من الدراسة هو التأكد من العلاقه بين الأمراض الانتهازية المختلفه والمضاعفات المرضيه في الاطفال المصابين بفيروس نقص المناعة البشرية –1 والحالة المناعية، عن طريق التقييم العددي لمجموعة التمايز (CD4) و نسبة خلايا CD4 **الطريقه:** أجريت هذه الدراسة خلال الفترة من يناير 2009 إلى يونيو 2010 في مركز العلاج المضاد للفيروسات في معهد شارما العالي للعلوم الطبية (روتاك)، وهومستشفى للرعاية الثالثه في شمال الهند. تمت الدراسه على 20 طفلا مصابا ب–HIV-1 تتراوح أعمارهم بين 4–157 أشهر. تم الحصول على المعلومات الديموغرافية والقياسات التشخيصية الأساسيه للأطفال المرضى قبل بدء العلاج الحثيث بالمضاد للفيروسات (HAART). أعطيت جرعة ثابتة من HAART اعتمادا على وزن المريض و تمت اعادة القيلسات بعد ستة أشهر من علاج HAART. **النتائج:** كانت هناك زيادة كبيرة في مستوى خضاب الدم والوزن والطول وعدد خلايا CD4 بعد ستة أشهر من العلاج ب HAART ولوحظ تحسن كبير ($P < 0.05$) في الحالة المناعية للمرضى وفقا لمنظمة الصحة العالمية **الخلاصة:** هذه الدراسة تشير الى أن شدة وتواتر المضاعفات الانتهازية في طب الأطفال المرضى مع HIV–1 ارتفع مع الانخفاض في تعداد CD4 . ان علاج العدوى الانتهازية، جنبا إلى جنب مع العلاج المضاد للفيروسات، قد يؤدي إلى الانتعاش السريري والمناعي وانخفاض معدل الإصابة بالالتهابات الانتهازية المستقبليه. ان أعداد CD4 قد تعطي فكرة أولية للطبيب المعالج عن الحالة المناعية لكل طفل ويمكن أيضا أن تستخدم كعلامة بيولوجية لفاعلية علاج HAART. يجب ضمان امتثال المريض خلال علاج HAART لأن هذا يشكل عاملا رئيسيا في تحسين النتائج.

مفتاح الكلمات: الإيدز؛ العلاج المضاد للفيروسات النشط للغاية؛ العدوى الانتهازية المرتبطه بالإيدز؛ عدد اللمفاويات CD4؛ الهند.

Advances in Knowledge

- *The results of this study show that while specific infections cannot be predicted based upon the cluster of differentiation 4 (CD4) count and percentage, the frequency and severity of opportunistic infections increase as the CD4 count falls.*
- *This study demonstrated that highly active antiretroviral therapy (HAART) has significant positive effects on the health of patients with human immunodeficiency virus (HIV)-1, including their weight and immunological status.*

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Application to Patient Care

- *This study supports the benefits of HAART in improving the nutritional status of patients, leading to decreased hospitalisation and a reduction in the healthcare costs associated with this. However, HAART is known to have high dropout rates and patient complicance must be emphasised in order to achieve the best results.*
- *As the results of this study demonstrate that specific infections cannot be predicted based upon the CD4 count alone, physicians should consistently test for opportunistic infections in HIV-1 patients. In resource-limited settings where CD4 count tests are not available, opportunistic infections may be used as a guide for the patient's immunological status and to inform the prescription of HAART.*

Acquired immune deficiency syndrome (AIDS) was first recognised in the USA in 1981 due to an unexplained *Pneumocystis jiroveci* infection in a previously healthy man. Ever since its appearance, the disease has progressed rapidly and is now being reported from every corner of the globe.[1] AIDS is caused by the human immune deficiency virus (HIV) types 1 and 2. The disease is present worldwide, but mainly concentrated in sub-Saharan Africa and Asia. The number of HIV patients in India is growing fast, and the total number of infected people in this country is second only to that in Africa.[1] There is an increasing number of paediatric HIV patients worldwide, with an estimated 2.5 million cases (7.5%) in 2007.[2] In the developed world, paediatric patients constitute 2% of the HIV-infected population, whereas in developing countries, 15–20% of the total HIV-infected population are children.[3] Ever since HIV was first registered in India in 1986, the country has reported a HIV seropositivity prevalence of 2.5%.[4] In India, AIDS is rapidly increasing among the HIV population; with the current rate of increase, India will soon have the highest AIDS prevalence worldwide.[4]

HIV is most commonly contracted through vertical transmission, as observed by Rogers *et al.* when investigating an American paediatric population.[5] Cluster of differentiation 4 (CD4) cells are the primary cellular target for HIV.[1] A diagnosis of HIV can be made by detecting viral antibodies, ribonucleic acid or antigens, for example by using a protein 24 antigen assay. The HIV deoxyribonucleic acid (DNA) polymerase chain reaction (PCR) test is the preferred virology assay among developed countries.[6]

Opportunistic infections occur in HIV-positive individuals as their immune system weakens and the aetiology of the infectious organism is affected by the immunological status of the patient.[7,8] In more economically developed countries, due to the extensive use of highly active antiretroviral therapy (HAART), infections like cytomegalovirus (CMV), cryptosporidiosis and toxoplasmosis predominate.[4,9] However, in developing countries, infections due to *Mycobacterium tuberculosis* are more common.[4,9]

The present study aimed to find a correlation between various HIV-related complications, both infectious and non-infectious, and immune status, measured by CD4 count. The study also examined changes in growth and opportunistic infections among patients after they began treatment with HAART.

Methods

This prospective study was undertaken in clinical and laboratory settings for an 18-month period between January 2009 and June 2010. A total of 20 paediatric patients with HIV-1 infections were observed. The subjects were either inpatients or outpatients at the Antiretroviral Treatment Centre of the Pt. B.D. Sharma Post Graduate Institute of Medical Sciences, a tertiary care hospital in Rohtak, Haryana, in northern India. All patients tested positive for HIV-1 infections confirmed by three positive enzyme-linked immunosorbent assay tests. To be included in the study, subjects also had to fulfil the World Health Organization (WHO) revised clinical staging system for HIV/AIDS in children,[10] and have one or more opportunistic infections or complications. Children under 18 months of age were excluded from the study, unless the diagnosis of HIV-1 had been confirmed by DNA PCR assay and they were already being treated for an opportunistic infection. The patients' clinical parameters prior to the beginning of the study were considered to be the control values.

All children enrolled in this study were followed-up monthly for six months. Clinical and nutritional examinations were done at each visit to identify any opportunistic infections. Each child underwent detailed general physical and systemic examinations at the time of their enrolment in the study. At this time, a record was made of their baseline data, including demographic details, nutritional status and malnutrition classification according to the Indian Academy of Pediatrics.[11] The possible modes of HIV transmission were also recorded via a thorough patient history, focusing on the patients' antenatal and perinatal history. Any suggestion of risk behaviours among the parents, the HIV status of the parents, if the patient had undergone previous blood transfusions and whether the patient had been breastfed were also documented. Laboratory investigations were

subsequently performed to estimate haemoglobin (Hb) concentration, absolute lymphocyte count, serum glutamic oxaloacetic transaminase to pyruvic transaminase ratio, serum creatinine and cholesterol. Additionally, all of the children underwent chest radiography and ultrasound abdomen scans. The CD4 count was measured using a fully automated fluorescence-activated cell sorting cytometer (S3™ Cell Sorter, Bio-Rad Laboratories, Inc., Hercules, California, USA). The CD4 percentage was calculated as follows: CD4 percentage = (absolute CD4 T-lymphocyte count divided by total lymphocyte count) x 100. The CD4 and absolute lymphocyte counts were repeated six months after their first visit.

Viral opportunistic infections like herpes *simplex*, herpes *zoster* and *Molluscum contagiosum* were identified clinically. Suspected bacterial and fungal infections were confirmed by culture, serology and Gram staining, or with special stains like Indian ink. The children were investigated for tuberculosis if they had any of the following symptoms: a fever and cough lasting more than one month; marked weight loss or *marasmus*; multiple lymphadenopathy; meningeal syndrome; a positive tuberculin skin test (with induration of more than 10 mm), or pulmonary infiltrates with or without lymph nodes as identified on a chest radiograph. Anthropometry and a detailed nutritional examination were performed to identify any signs of wasting. Smear testing and cultures were performed on samples obtained from the subjects by early gastric aspiration after overnight fasting for three consecutive days. Patients with a history of oral thrush or other fungal infections underwent fungal swab testing and cultures using axillary, inguinal, rectal, throat swabs, urine (for fungal *hyphae*) and blood samples. Patients with persistent diarrhoea underwent three stool examinations and cultures to identify the microbes responsible for this symptom.

All of the patients enrolled in the study were graded according to the revised WHO clinical staging system for HIV/AIDS in children.[10] Immunological staging was determined according to the WHO classifications of HIV-associated immunodeficiency in infants and children.[8] Children who were in clinical stages 3 or 4 were candidates for HAART. Children who were in stages 1 and 2 were also candidates if they fulfilled the following criteria according to their age group: <11 months (if CD4 <1,500 cells/mm^3 or <25%); 12–35 months (if CD4 <750 cells/mm^3 or <20%); 36–59 months (if CD4 <350 cells/mm^3 or <15%), or >5 years (if CD4 <200 cells/mm^3).

If applicable, patients were prescribed a dispersible fixed-dose combination of HAART, containing stavudine (30 mg or 40 mg), lamivudine (150 mg) and nevirapine (200 mg). These drugs were provided free of charge from the Antiretroviral Treatment Centre at the Pt. B.D. Sharma Post Graduate Institute of Medical Sciences. Patients with tuberculosis were started on HAART after the completion of antitubercular therapy (ATT); in severely immunosuppressed patients, HAART was prescribed only after 2–8 weeks of ATT. Opportunistic infections were treated according to guidelines issued by the National AIDS Control Organization for HIV care and treatment for infants and children in India.[7]

In this study, analysed variables included the child's nutritional status, absolute lymphocyte count, CD4 percentage, CD4 count and any changes in this count, as well as the child's immune status after undergoing specific therapy for various opportunistic infections and complications. All the data were inserted in patient proformas. The statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS), Version 16 (IBM, Corp., Chicago, Illinois, USA). The student's t-test and Fisher's exact test were applied to the data.

Before each child was enrolled in the study, informed consent was obtained from their parents. In cases where the patient had lost both parents, consent was obtained from the child's guardians. The study was approved by the Ethical & Scientific Committee of the Pt. B.D. Sharma Post Graduate Institute of Medical Sciences in December 2008.

Results

A total of 20 paediatric patients with HIV-1 infections ranging in age from 4 to 157 months were observed during the study period, with a mean age at presentation of 72.4 ± 46.18 months. None of the patients dropped out of the study during the study period. Among the children, the predominant mode of HIV transmission was vertical. A total of 50% of the patients had experienced the loss of one or both parents. This posed great difficulty in maintaining the care of these patients, especially as it was not always clear who was responsible for them. A total of 19 patients (95%) were prescribed HAART; of these, 95% were compliant with the treatment and did not miss any doses. One patient (5.3%) developed a skin rash following the commencement of HAART; however, the rash subsided without treatment and the patient was able to continue the therapy for the required duration.

At the beginning of the study, the majority of the patients (75%) suffered from wasting, with a weight-for-age measurement in the <3rd percentile, as per the

Table 1: Weight-for-age and height-for-age percentiles of human immunodeficiency virus-1-infected children before and after six months of highly active antiretroviral therapy (N = 20)

Percentile[12]	Weight-for-age* in kg		Height-for-age* in cm	
	Before therapy	After therapy	Before therapy	After therapy
<3rd	15	4	4	3
3–10th	4	3	5	1
10–50th	1	11	9	15
>50th	0	2	2	1

**Divided by the number of patients.*

Table 3: Comparison of clinical parameters, immunological markers, World Health Organization clinical stage and immunological stage for human immunodeficiency virus-1-infected children at diagnosis and following six months of highly active antiretroviral therapy and treatment for opportunistic infections (N = 20)

	At diagnosis*	After therapy	*P* value
Clinical parameter			
Mean Hb in g %	7.78 ± 2.36	9.28 ± 1.13	<0.05
Mean weight in kg	13.95 ± 7.63	18.8 ± 7.32	<0.01
Mean height in cm	105.72 ± 27.06	110 ± 25.66	<0.01
Immunological marker			
Mean total lymphocyte count in mm^3	3,061 ± 1,723	2,931 ± 1,169	>0.05
Mean CD4 count in cells/mm^3	391 ± 274	720 ± 356	<0.01
Mean CD4 percentage	12.86 ± 7.54	25.53 ± 15.03	<0.01
Clinical stage[10]			
1	0	0	<0.05
2	1	17	
3	12	1	
4	7	2	
Immunological stage[8]			
Not significant	2	10	<0.05
Mild	1	6	
Advanced	8	3	
Severe	9	1	

Hb = haemoglobin; CD4 = cluster of differentiation 4.
**Upon diagnosis of an opportunistic infection.*

Table 2: Clinical manifestations of opportunistic infections or complications among human immunodeficiency virus-1-infected children (N = 20)

Manifestation	n	%
Fever	19	95
Cough	11	55
Diarrhoea	8	40
Generalised weakness/anorexia	19	95
Weight loss/failure to thrive	17	85
Abdomen distension	5	25
Generalised lymphadenopathy	15	75
Hepatomegaly	14	70
Splenomegaly	6	30
Other		
Skin lesions	3	15
Otitis	3	15
Oral thrush	4	20
Chelosis/apthous ulcer/stomatitis	6	30
Seizures	5	25
Pleural effusion	1	5

Centers for Disease Control and Prevention (CDC) growth charts.[12] However, 45% of the patients had a length-for-age measurement that was between the 10th and 50th percentiles, which demonstrated that wasting was more prevalent in HIV patients than stunted growth [Table 1]. Fevers, anorexia and weakness were the most common symptoms noticed in the study (95%), followed by weight loss/failure to thrive (85%) [Table 2].

Following six months of HAART, a significant increase was noted in the mean Hb, height and weight measurements of the HIV-1-infected children. Upon admission to the study, the mean Hb was 7.78 ± 2.36 g %, mean weight was 13.95 ± 7.63 kg and mean height was 105.72 ± 27.06 cm. Following HAART, these values increased to 9.28 ± 1.13 g % (P <0.05), 18.8 ± 7.32 kg (P <0.01) and 110 ± 25.66 cm (P <0.01), respectively. These results suggest that the treatment for opportunistic infections and HAART significantly improved clinical outcomes among the patients [Table 3]. Additionally, the rate of hospital admissions decreased during the study period. A total of 16 patients (80%) were hospitalised at the beginning of the study, but only 3 (15%) were admitted to the hospital during the six-month follow-up period, demonstrating that combined therapy for opportunistic infections and HAART were effective in decreasing the hospital admission rate for this group of HIV-positive children.

Table 4: Comparison of the clinical parameters of human immunodeficiency virus-1-infected children at diagnosis and following six months of highly active antiretroviral therapy by age group and the two most common opportunistic infections (N = 20)

Clinical parameter	Age group					
	<60 months			>60 months		
	Before therapy	After therapy	*P* value	Before therapy	After therapy	*P* value
Mean weight in kg	8.05 ± 3.30	13.1 ± 2.56	<0.01	20.05 ± 5.55	25.5 ± 5.88	<0.01
Mean height in cm	83.55 ± 16.83	88.85 ± 15.06	<0.01	127.9 ± 12.5	131.25 ± 12.81	<0.01
Mean CD4 count in cells/mm^3	544 ± 286	910 ± 348	<0.05	238 ± 158	550 ± 293	<0.05
Mean CD4 percentage	16.84 ± 7.99	28.25 ± 8.23	<0.05	11.5 ± 9.06	23.94 ± 11.58	<0.05
	Tuberculosis patients					
	At diagnosis		After therapy		*P* value	
Mean Hb in g/dL	8.08 ± 1.88		9.83 ± 1.50		<0.05	
Mean weight in kg	16.5 ± 9.54		21.08 ± 10.18		<0.01	
Mean height in cm	112.67 ± 22.21		116.33 ± 21.79		<0.01	
	Oral thrush patients					
	At diagnosis		After therapy		*P* value	
Mean Hb in g/dL	5.9 ± 0.93		8.45 ± 0.70		<0.05	
Mean weight in kg	11 ± 6.48		16.3 ± 6.54		>0.05	
Mean height in cm	102 ± 25.39		106 ± 23.51		<0.05	

CD4 = cluster of differentiation 4; Hb = haemoglobin.

There was a significant increase in CD4 count and CD4 percentage among the patients after six months of HAART, as well as a statistically significant (P <0.05) change in clinical stage. The majority of patients initially categorised as stages 3 and 4 were subsequently reclassified to stage 2 following treatment. An improvement in immunological stage was also observed, with the majority of patients promoted to the 'not significant' group after six months of treatment [Table 3].

Furthermore, the results showed a statistically significant improvement in the mean weight, height, CD4 count and CD4 percentage of patients when the data was compared by age group (<60 *versus* >60 months). Additionally, there was a significant improvement in weight, height and Hb among tuberculosis patients following six months of HAART and a significant increase in Hb and height among patients with oral thrush [Table 4]. The most commonly observed opportunistic infections in this study were tuberculosis and thrush. Other opportunistic infections seen included persistent diarrhoea, bacteremia, meningitis, HIV encephalopathy, disseminated CMV and systemic thrush [Table 5]. At the time of presentation, eight (40%) patients had persistent diarrhoea and 11 (55%) patients had a cough.

The distribution of various opportunistic infections were noted in relation to the patients' immunological status. It was observed that six patients (30%) had CD4 counts <200 cells/mm^3 and 14 (70%) had CD4 counts >200 cells/mm^3. Similarly, eight patients (40%) had a CD4 percentage <20% and 12 (60%) had a CD4 percentage >20%. Infections occurring with CD4 counts <200 cells/mm^3 included tuberculosis (33.3%), oral thrush (25%), persistent diarrhoea (25%), bacteremia (25%) and disseminated CMV [Table 6].

Discussion

The global AIDS epidemic is one of the greatest challenges facing the current generation and the extent of this problem among children is growing rapidly. The aim of this study was to determine the correlation between opportunistic infections and complications among HIV-1-infected children and immune status, evaluated by CD4 count and percentage.

Upon enrolment in the present study, the majority of the patients had a weight-for-age below the 3rd percentile and 20% had a height-for-age below the 3rd percentile. Another 20% and 25% of the patients were between the 3rd and 10th percentiles, for weight- and height-for-age, respectively. Only two patients had a

Table 5: Distribution of opportunistic infections or complications observed among human immunodeficiency virus-1-infected children by mean age at presentation and immunological indicators (N = 20)

Opportunistic infection/complication	n	Mean age in months	Mean total lymphocyte count in mm^3	Mean CD4 count in mm^3 (range)	Mean CD4 percentage (range)
Tuberculosis	6	78 ± 42.76	3,391 ± 2,535	333 ± 302 (21–839)	9.2 ± 6.88 (2.9–21.4)
Oral thrush	4	60.2 ± 48.91	3,041 ± 1,680	541 ± 296 (98–716)	17.46 ± 3.82 (14.1–21.78)
Persistent diarrhoea	3	88.33 ± 66.6	3,765 ± 880	521 ± 406 (171–967)	15.76 ± 15.71 (4.4–33.7)
Bacteremia	3	72 ± 54.99	2,481 ± 2,002	356 ± 280 (98–654)	14.4 ± 0.30 (14.1–14.7
Meningitis	2	66 ± 42.42	2,171 ± 612	295 ± 9.19 (289–302)	14.10 ± 3.53 (11.6–16.6)
HIV encephalopathy	2	91 ± 76.36	3,605 ± 1,391	3,322 ± 178 (206–458)	11.24 ± 1.78 (9.98–12.5)
Disseminated CMV	1	4	467	19	4
Systemic thrush	1	96	3,268	510	15.6

CD4 = cluster of differentiation 4; CMV = cytomegalovirus.

height-for-age above the 50th percentile, while none of the patients had a weight-for-age above the 50th percentile. These results are similar to those found among other studies based in India, with Lodha *et al.* describing growth failure among all of the observed paediatric HIV-infected patients (n = 27) in their study and Shah reporting weight loss and failure to thrive in 35.6% out of 317 children with HIV infections.[13,14]

In this study, fever, anorexia and weakness were the most commonly observed symptoms, followed by weight loss/failure to thrive. Furthermore, a sizeable percentage of the patients had persistent diarrhoea and a cough at the time of presentation. Similar manifestations of HIV infection have also been reported elsewhere in India. Verghese *et al.* studied the clinical manifestations of HIV infection among 88 children and reported hepatomegaly in 72%, lymphadenopathy in 60%, splenomegaly in 43%, failure to thrive in 58%, recurrent diarrhoea in 36%, *M. contagiosum* in 4% and pulmonary tuberculosis in 14%.[15] Dhurat *et al.* noted non-specific presenting symptoms in 41 children with perinatally transmitted

Table 6: Distribution of various opportunistic infections or complications among human immunodeficiency virus-1-infected children in relation to clinical markers of immune status (N = 20)

Opportunistic infection/complication		CD4 count <200 cells/mm^3	CD4 count >200 cells/mm^3	CD4 percentage <20%	CD4 percentage >20%
Tuberculosis	Yes	2	4	4	2
	No	4	10	4	10
Thrush	Yes	-	1	1	-
	No	6	13	7	12
Oral thrush	Yes	1	3	2	2
	No	5	11	6	10
Persistent diarrhoea	Yes	1	2	2	1
	No	5	12	6	11
Bacteremia	Yes	1	2	3	0
	No	5	12	5	12
HIV encephalopathy	Yes	0	2	2	0
	No	6	12	6	12
Meningitis	Yes	0	2	2	0
	No	6	12	6	12
Disseminated CMV	Yes	1	-	1	-

CD4 = cluster of differentiation 4; CMV = cytomegalovirus.

HIV, such as tuberculosis (67.5%), failure to thrive (48.6%), hepatomegaly (51.9%), splenomegaly (48.6%), lymphadenopathy (35%), recurrent fever (29.3%) and diarrhoea (27.2%).[16] Among 58 HIV-infected children, Madhivanan *et al.* reported the following manifestations: oral thrush (43%), tuberculosis (43%), hepatosplenomegaly (14%), lymphadenopathy (14%), papulopruritic dermatitis (10%) and chronic diarrhoea (7%).[17] These manifestations and infections have a very high incidence in AIDS patients and it is advisable that paediatric patients presenting with one or more of these conditions be evaluated for the syndrome.

Among the patient population of the current study, significant increases in mean Hb, weight and height were noted by the end of the study, following treatment with HAART. Consequently, it can be inferred that the combined treatment of opportunistic infections and HAART significantly improved the patients' clinical outcomes. Similar results were reported in studies conducted by Puthanakit *et al.* in Thailand and Wamalwa *et al.* in Kenya.[18,19] Additionally, there was a significant increase in the mean total lymphocyte count, CD4 count and CD4 percentage after the six-month follow-up and treatment period of the current study. Puthanakit *et al.* identified that mean CD4 percentage increased to 21%, from a baseline of 3%, while CD4 count increased from 126 to 532 cells/mm^3 after 72 weeks of HAART.[18] Wamalwa *et al.* and Pensi also recorded significant increases in CD4 count after their patients underwent antiretroviral therapy.[19,20]

The HIV-infected children in the present study were categorised into four clinical stages using the WHO criteria.[10] There was a statistically significant (P <0.05) change in clinical stage after six months of therapy. Several studies have reported comparable improvements in clinical stage after antiretroviral therapy.[20,21] The change in immunological classifications for the current patient population after six months of therapy was also found to be statistically significant. This indicates that an immunological improvement is seen in HIV-infected children after treatment with HAART. Similar results were reported by Natu *et al.* while studying the effectiveness of HAART in relation to CDC immunological classifications.[21]

In the present study, the predominant opportunistic infections were tuberculosis and oral thrush. Van Dyke reported that the most common opportunistic infections among children with HIV included serious bacterial infections (pneumonia and bacteremia), pneumocystis pneumonia, tuberculosis, non-tuberculous mycobacterial infections and CMV.[22] Tuberculosis was the most commonly occurring opportunistic infection in Indian children in two separate studies.[14,17] Balkhair *et al.* found that *Pneumocystis jiroveci* pneumonia was the most frequently observed opportunistic infection among their study population, followed by cryptococcal meningitis, CMV retinitis, disseminated tuberculosis and cerebral toxoplasmosis.[23]

Among the studied HIV-infected paediatric population, 30% had a CD4 count <200 cells/mm^3 and 40% had a CD4 percentage <20% upon diagnosis of an opportunistic infection. The Swiss HIV Cohort Study reported that the risk of developing opportunistic infections is increased by 2.5 if the CD4 count is between 51–200 cells/mm^3; this risk increases to 5.8 for counts <50 cells/mm^3 in comparison to those >200 cells/mm^3.[24] Various studies have demonstrated that lower CD4 counts are associated with an increased prevalence of opportunistic infections.[13,25,26] While these infections can occur at any CD4 count, the severity and frequency of opportunistic infections increases when the count falls below 200 cells/mm^3. The organisms responsible for causing opportunistic infections also differ with changes in immune status. A hierarchy of severity can therefore be proposed for opportunistic infections in relation to CD4 values as follows: oral thrush <tuberculosis <disseminated CMV infection.

The mean CD4 count and percentage among tuberculosis patients at the time of diagnosis was 333 cells/mm^3 and 9.2%, respectively, while for the patients with oral thrush it was 541 cells/mm^3 and 17.46%, respectively. Similar results were found by Laufer *et al.* and Shah.[14,27] The Swiss HIV Cohort Study found a median CD4 count of 6 cells/mm^3 in patients newly-diagnosed with tuberculosis, while those with a diagnosis of CMV had a median CD4 count of 28 cells/mm^3.[24] The above studies reported a variety of CD4 counts and percentages upon diagnosis of different opportunistic infections. This makes it difficult to define specific CD4 count cut-off values for different opportunistic infections.

Individual opportunistic infections were analysed separately for changes to the patients' immunological and clinical profiles after six months of HAART and treatments for the relevant opportunistic infection. All of the studied patients experienced a gain in both weight and height along with an increase in their CD4 count. Guillén *et al.* reported that there was a significant decrease in HIV-related complications (infectious as well as non-infectious) among their study population after the introduction of HAART.[28] The authors also found that patients who benefited from HAART had higher CD4 counts than those who had not yet been treated; furthermore, those who were not treated with HAART had a 2.77% incidence of wasting syndrome,

compared to an incidence of only 0.24% among those who underwent the treatment.[28] Similar results were found by Puthanakit *et al*.[18]

All but one of the patients in the current study were prescribed HAART and the vast majority (95%) of these children were compliant with the treatment requirements. Nischal *et al*. suggested that the minimum adherence to ART should be 95%.[29] As with the present study, minor skin rashes have been observed on some patients during the first two weeks of treatment with nevirapine, which then subsided completely.[30] In the same cohort, one child developed pancreatitis while on HAART, which improved with conservative management. No other significant side-effects were noticed.[30]

In developing countries like India, it is common for paediatric patients without HIV infections to present with certain health concerns, including a failure to thrive, severe acute malnutrition, lower respiratory infections and gastrointestinal problems (such as recurrent diarrhoea). Unfortunately, these symptoms are also frequently seen among children with HIV infections. These overlapping symptoms make it difficult to ensure an early diagnosis of HIV, often leading to a delay in the start of treatment. This raises a dilemma as to whether all children presenting with such complaints should be screened for HIV. Nevertheless, it is prudent to conduct an HIV screening for children presenting with severe malnutrition, recurrent or persistent diarrhoea, recurrent pneumonia, disseminated tuberculosis (like tubercular meningitis) or pulmonary tuberculosis. Consequently, this will allow the early allocation of treatment and significantly improve patient outcomes.

These findings should be interpreted with caution as the study was subject to certain limitations, including the relatively small sample size and the short duration of follow-up. Further studies of longer duration and with a larger cohort are recommended.

Conclusion

Among 20 HIV-infected children, increased Hb, weight, height and CD4 cell counts were observed following six months of treatment with HAART. Improvements in clinical stage and immunological status were also observed among the patients. The most frequently noted opportunistic infections were tuberculosis followed by oral thrush. The severity and frequency of opportunistic infections increased with a decrease in the CD4 count. Treatment of opportunistic infections, in addition to HAART, led to both clinical and immunological recovery, as well as a decreased incidence of future opportunistic infections. These findings indicate that healthcare professionals may be able to use a patient's CD4 count as one of the first indications of their immunological status, in association with an understanding of the opportunistic infection in question. The CD4 count could also potentially be used as a biological marker of HAART efficacy. However, compliance to HAART must be ensured as it is a key factor in achieving optimal results from this type of treatment.

CONFLICT OF INTEREST

The authors report no conflicts of interest. No funding was received during the completion of this study.

References

1. Malkani M. Pediatric HIV disease. In: Parthasarathy A, Menon PSN, Eds. IAP Textbook of Pediatrics. 4th ed. New Delhi, India: Jaypee Brothers Medical Publishers Ltd., 2009. Pp. 414–18.
2. UNAIDS. AIDS epidemic update: December 2007. From: www.data.unaids.org/pub/epislides/2007/2007_epiupdate_en.pdf Accessed: Aug 2014.
3. Newell ML, Peckham CS. HIV infection in Europe. In: Pizzo P, Wilfert CM, Eds. Pediatric AIDS: The challenge of HIV infection in infants, children, and adolescents. 2nd ed. Baltimore, Maryland, USA; Lippincott William & Wilkins, 1994. Pp. 21–30.
4. Merchant RH, Oswal JS, Bhagwat V, Karkare J. Clinical profile of HIV Infection. Indian Pediatr 2001; 38:239–46.
5. Rogers MF, Caldwell MB, Gwinn ML, Simonds RJ. Epidemiology of pediatric human immunodeficiency virus infection in the United States. Acta Pediatr Suppl 1994; 400:5–7. doi: 10.1111/j.1651-2227.1994.tb13324.x.
6. Yogev R, Chadwick EG. Acquired immunodeficiency syndrome (human immunodeficiency virus). In: Kliegman RM, Behrman RE, Jenson HB, Stanton BF, Eds. Nelson Textbook of Pediatrics. 18th ed. Philadelphia, Pennsylvania, USA: Elsevier Saunders, 2007. Pp. 1427–42.
7. National Aids Control Organisation. Guidelines for HIV Care and Treatment in Infants and Children. From: www.naco.gov.in/upload/Policies%20&%20Guidelines/4-%20Guidelines%20for%20HIV%20care%20and%20treatment%20in%20Infants%20and%20children.pdf Accessed: Aug 2014.
8. World Health Organization. Antiretroviral therapy of HIV infection in infants and children in resource limited settings: Towards universal access - Recommendations for a public health approach, 2006. From: www.who.int/hiv/pub/guidelines/WHOpaediatric.pdf Accessed: Feb 2014.
9. Bhargava A, Singh DK, Rai R. Sero-prevalence of viral co-infections in HIV infected children of Northern India. Indian J Pediatr 2009; 76:917–9. doi: 10.1007/s12098-009-0142-x.
10. World Health Organization. WHO Case Definitions of HIV for Surveillance and Revised Clinical Staging and Immunological Classification of HIV-related Disease in Adults and Children. From: www.who.int/hiv/pub/guidelines/HIVstaging150307.pdf Accessed: Jan 2014.
11. Mantan M, Bagga A. Nutrition and Nutritional Disorders. In: Kabra SK, Srivastava SN, Eds. Pediatrics: A Concise Text. New Delhi, India: Reed Elsevier Private Ltd., 2011. Pp. 47–60.
12. Centers for Disease Control and Prevention. Growth Charts. From: www.cdc.gov/growthcharts/ Accessed: Apr 2014.

13. Lodha R, Singhal T, Jain Y, Kabra SK, Seth P, Seth V. Pediatric HIV infection in tertiary care center in north India: Early impression. Indian Pediatr 2000; 37:982–6.

14. Shah I. Age related clinical manifestations of HIV infection in Indian children. J Trop Pediatr 2005; 51:300–3. doi: 10.1093/tropej/fmi018.

15. Verghese VP, Cherian T, Cherian AJ, Babu PG, John TJ, Kirubakaran C, et al. Clinical manifestations of HIV-1 infection. Indian Pediatr 2002; 39:57–63.

16. Dhurat R, Manglani M, Sharma R, Shah NK. Clinical spectrum of HIV infection. Indian Pediatr 2000; 37:831–6.

17. Madhivanan P, Mothi SN, Kumarasamy K, Yepthomi T, Venkatesan C, Lambert JS, et al. Clinical manifestations of HIV infected children. Indian J Pediatr 2003; 70:615–21. doi: 10.1007/BF02724249.

18. Puthanakit T, Oberdorfer A, Akarathum N, Kanjanavanit S, Wannarit P, Sirisanthana T, et al. Efficacy of highly active antiretroviral therapy in HIV-infected children participating in Thailand's National Access to Antiretroviral Program. Clin Infect Dis 2005; 41:100–7. doi: 10.1086/430714.

19. Wamalwa DC, Farquhar C, Obimbo EM, Selig S, Mbori-Ngacha DA, Richardson BA, et al. Early response to highly active antiretroviral therapy in HIV-1-infected Kenyan children. J Acquir Immun Defic Syndr 2007; 45:311–17. doi: 10.1097/QAI.0b013e318042d613.

20. Pensi T. Fixed dose combination of lamivudine, stavudine and nevirapine in the treatment of pediatric HIV infection: A preliminary report. Indian Pediatr 2007; 44:519–21.

21. Natu SA, Daga SR. Antiretroviral therapy in children: Indian experience. Indian Pediatr 2007; 44:339–43.

22. Van Dyke RB. Opportunistic infections in pediatric HIV disease. Ann N Y Acad Sci 1993; 693:158–65.

23. Balkhair AA, Al-Muharrmi ZK, Ganguly S, Al-Jabri AA. Spectrum of AIDS defining opportunistic infections in a series of 77 hospitalised HIV-infected Omani patients. Sultan Qaboos Univ Med J 2012; 12:442–8.

24. Ledergerber B, Egger M, Erard V, Weber R, Hirschel B, Furrer H, et al. AIDS-related opportunistic illnesses occurring after initiation of potent antiretroviral therapy: The Swiss HIV Cohort Study. JAMA 1999; 282:2220–6. doi: 10.1001/jama.282.23.2220.

25. Moore RD, Chaisson RE. Natural history of opportunistic disease in an HIV-infected urban clinical cohort. Ann Intern Med 1996; 124:633–42. doi: 10.7326/0003-4819-124-7-199604010-00003.

26. 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; 45:140–3.

27. Laufer MK, van Oosterhout JJ, Perez MA, Kanyanganlika J, Taylor TE, Plowe CV, et al. Observational cohort study of HIV-infected African children. Pediatr Infect Dis J 2006; 25:623–7. doi: 10.1097/01.inf.0000220264.45692.a0.

28. Guillén S, García San Miguel L, Resino S, Bellón JM, González I, Jiménez de Ory S, et al. Opportunistic infections and organ-specific diseases in HIV-1-infected children: A cohort study (1990-2006). HIV Med 2010; 11:245–52. doi: 10.1111/j.1468-1293.2009.00768.x.

29. Nischal KC, Khopkar U, Saple DG. Improving adherence to antiretroviral therapy. Indian J Dermatol Venereol Leprol 2005; 71:316–20. doi: 10.4103/0378-6323.16780.

30. Lodha R, Upadhyay A, Kabra SK. Antiretroviral therapy in HIV-1 infected children. Indian Pediatr 2005; 42:789–96.