ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS) IN MULTI-TRANSFUSED CHILDREN WITH THALASSEMIA

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ABSTRACT

Two hundred and three multi-transfused children with thalassemia attending the Thalassemia Clinic of the Charak Palika Hospital, New Delhi were screened for human immunodeficiency virus (HIV) antibodies by ELISA and all positive cases were confirmed by Western Blot. Of the 203 children screened, 18 (8.9%) were HIV positive, and in these children a detailed immunological work up was done and compared to 18 age-matched HIV negative thalassemics as controls. The tests included absolute lymphocyte counts (ALC), absolute and percentages of CD4+ and CD8+ cells and their ratios (CD4/CD8), immunoglobulin levels (IgG, IgM and IgA) and delayed cutaneous hypersensitivity (DCH) test by Multitest CMI in all the cases and the controls.

Of the 18 HIV positive children, 6 were diagnosed to have clinical AIDS as per the WHO criteria. After immunological testing, the children were further classified according to the CDC criteria. By these criteria, 11 children were classified as P1 A (asymptomatic infection, normal immune function), 1 child as P1 B (asymptomatic infection, abnormal immune function), 2 children as P2 A (symptomatic infection with non-specific findings), 1 child as P2 C (lymphocytic interstitial pneumonitis), 1 child as P2 D1 (Pneumocystis carinii pneumonia) and 2 children as P2 D2 (symptomatic infection with infections).

In this paper, the clinical features of the children with AIDS is described, and the immunologic functions of these children are compared with the HIV positive asymptomatic children and with controls. These are the first cases of AIDS in the pediatric age group from India.

Key words: *AIDS, Human immunodeficiency virus, Transfusion.*

Human immunodeficiency virus (HIV) infection was detected for the first time in India among prostitutes in Tamil Nadu (Southern India) in April 1986 and the first HIV positive blood donor in July 1987. Since then, an increasingly large number of paid donors have been found to be seropositive in many cities and towns in different parts of the country. The Indian Council of Medical Research (ICMR) AIDS Task Force in 1988, therefore, recommended donor screening in metropolitan

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cities(1). Since then, the ICMR sero-surveillance data has shown an ELISA positivity of between 0.1-1.5% in donated blood from different parts of the country(1,2).

The New Delhi Municipal Committee's Charak Palika Hospital is a major transfusion centre in Northern India. Here about 200 patients of thalassemia are registered, of whom 10-12 are transfused daily. Since most of these children had received many units of blood before routine donor screening was started, a systematic screening of these children for HIV infection was undertaken. All children found to be seropositive were further evaluated for their immunologic status. The present manuscript describes the results of this study.

Material and Methods

Two hundred and three multi-transfused children of thalassemia and related hemoglobinopathies attending the Thalassemia Clinic of the Charak Palika Hospital were screened for HIV antibodies. The HIV testing was carried out in two stages:

(*i*) *Screening by ELISA:* Sera from all the multi-transfused children were screened for HIV antibodies by competitive ELISA using recombinant antigens coded by *gag* and *env* genes as solid phase (Wellcozyme Recombinant, Welcome Diagnostics, UK).

(*ii*) *Western blot (WB):* WB for confirmatory testing of HIV antibody was carried out using commercial reagents according to the manufacturer's instructions (Du Pont, USA). In each run of test samples, positive, negative and cut-off controls were included. Interpretation of WB positivity was made by Centres for Disease Control (CDC) criteria(3) as well as by WHO criteria(4).

All WB positive cases were worked-up immunologically as follows:

(*i*) *Absolute lymphocyte counts (ALC):* This was done by the standard mothod. An ALC below 1500/mm^3 was considered to signify lymphopenia and immunodeficiency.

(*ii*) *Absolute and percentages of CD4+ and CD8+ lymphocyte counts:* CD4+ and CD8+ cell counts were done by the technique of Erber *et al.*(5), standardized earlier (Dakopatts, Denmark). A CD4+ count below 400/mm^3 and a CD4/CD8 ratio of less than 1 was considered abnormal.

(*iii*) *Immunoglobulins:* IgG, IgM and IgA were estimated in the serum by the technique of single radial immunodiffusion (Immunodiagnostics, Delhi, India).

(*iv*) *Delayed type cutaneous hypersensitivity (DCH):* DCH was recorded using 'Multitest CMI' kit (Institut Merieux, Lyon, France). Briefly, this consists of a disposable multipuncture device, preloaded with a battery of 7 common recall antigens, which are simultaneously injected intradermally on the forearm, and the indurations read after 48 hours. An individual induration of 2 mm or more was considered positive, and the sum of all the positive tests, if over 10 mm was considered to signify adequate immunity.

All the above immunologic tests were also carried out on a group of 18 age-matched HIV negative thalassemics, who acted as the controls.

Satistical analysis was done by the Kruskal-Wallis one way analysis of non-parametric values test.

Results

Of the 203 children screened for HIV antibodies, 184 had thalassemia major, 9 had beta thalassemia-HbE disease and 10 were transfusion dependent thalassemia intermedia. Eighteen (8.9%) of these children were detected to be HIV positive by

WB both by CDC and WHO criteria since all of them demonstrated p24, gp41 and gp120/160. Their ages ranged from 5.5-19.5 years (mean 9.67±3.07 years), and there were 11 males and 7 females. All the children had received unscreened blood prior to the start of routine donor screening, and in addition, 16 of the 18 children had a history of receiving at least 1 unit of blood from a paid donor in the past.

Of the 18 HIV positive children, 6 were diagnosed to have clinical AIDS according to the WHO criteria(6). The clinical features of these children are shown in *Table I.* After immunologic testing, these children were further classified according to the CDC criteria for children(7). Immunodeficiency was considered to be present when at least 2 of the 4 criteria were fulfilled, *i.e.,* 'Multitest CMI' reading less than 10 mm, CD4+ cell count less than 400/mm^3, ALC less than 1500/mm^3 and CD4/CD8 ratio less than 1. By these criteria, 11 children were classified as P1 A (asymptomatic infection, normal immune function), 1 child as P1 B (asymptomatic infection, abnormal immune function), 2 children as P2 A (symptomatic infection, abnormal immune function), 1 child each as P2 C (lymphocytic interstitial pneumonitis) and P2 D1 (*Pneumocystis carinii* pneumonia) and 2 children as P2 D2 (symptomatic infection with bacterial infections). It may be noted that 3 of the 6 children had thrombocytopenia, epistaxis and generalized purpuric lesions on the body.

TABLE I—*Clinical Features of the 6 Children with AIDS*

Feature	Frequency
Lymphadenopathy	6/6 (100)
Increased transfusion requirements	6/6 (100)
Prolonged diarrhea	4/6 (67)
Weight loss (more than 10%)	4/6 (67)
Prolonged pyrexia (more than 1 month)	4/6 (67)
Epistaxis, thrombocytopenia and purpura	3/6 (50)
Oropharyngeal candidiasis	3/6 (50)
Alopecia	2/6 (33)
Clubbing	1/6 (17)
Salivary gland enlargement	1/6 (17)
Lymphocytic interstitial pneumonitis (LIP)	1/6 (17)
Pneumocystis carinii pneumonia	1/6 (17)

Figures in parentheses indicate percentages

The results of the immunological tests in the 3 groups, 6 children with AIDS (Group A), 12 children with asymptomatic HIV infection (Group B) and 18 HIV negative children with thalassemia (Group C) are shown in *Table II.* ALC was not different in the 3 groups. CD4+ cell counts were lowest in Group A and they differed significantly from the other groups (Group A : Group B, $p<0.01$; Group A : Group C, $p<0.05$). The CD4/CD8 ratio in Group A was significantly lower than that of Group B ($p<0.05$) and Group C ($p<0.01$). The immunoglobulin values IgG, IgM and IgA were similar in all the 3 groups.

The numbers and percentages of children with deficiencies of individual immunologic parameters is shown in *Table III.* An abnormal 'Multitest CMI' reading was the most frequent abnormality in Group A and was seen in all the children of this group, as compared to 2/12 (16.6%) in Group B and in none in Group C. Abnormal CD4/CD8 ratios and low CD4+ cell counts were seen in 5/6 (83.3%). and 4/6 (66.6%), respectively in Group A, and abnormal ALC was the least frequent finding seen in only 2/6 (33.3%) cases. Overall,

TABLE II–*Results of Immunologic Tests in the 3 Groups*

Group	ALC/mm³	CD4+/mm³	CD4/CD8	Multitest CMI (mm)	IgG (mg/dl)	IgM (mg/dl)	IgA (mg/dl)
Group A (AIDS) (n=6)	1566±495 (766-2052)	408±284 (152-1005)	0.65±0.22 (0.14-1.1)	3.06±2.7 (0-9)	284±40 (225-316)	166±7 (155-176)	184±76 (66-301)
Group B (HIV +ve (Asymptomatic) (n=12)	6189±4270 (2544-15008)	1431±664 (514-2495)	1.56±2.58 (0.29-10)	13.66±6.17 (8-30.6)	350±118 (219-691)	213±61 (127-328)	177±45 (88-247)
Group C (Controls) (n=18)	5154±3120 (2257-13612)	1666±1303 (446-5444)	1.61±0.36 (0.55-2.11)	18.8±6.4 (11-33)	257±124 (188-305)	279±137 (166-318)	158±56 (96-256)
Statistical values							
A : B	NS	p<0.01	NS	p<0.05	NS	NS	NS
A : C	NS	p<0.05	p < 0.05	p<0.01	NS	NS	NS

TABLE III–*Numbers and Percentages of Children with Deficiencies in Individual Immunologic Parameters*

Groups	ALC <1500/mm³	CD4+ <400/mm³	CD4/CD8 <1	Multitest CMI <10 mm	Immune deficiency*
Group A (AIDS) (n=6)	2/6 (33.3)	4/6 (66.6)	5/6 (83.3)	6/6 (100)	6/6 (100)
Group B (HIV +ve asymptomatic) (n=12)	0/12 (0)	0/12 (0)	7/12 (58.3)	2/12 (16.6)	1/12 (8.3)
Group C (Controls) (n=18)	0/18 (0)	0/18 (0)	0/18 (0)	0/18 (0)	0/18 (0)

* Immune deficiency: at least 2 of the 4 immunologic parameters abnormal;
Figures in parantheses express percentages.

the 6 children of Group A were classified as being immunodeficient as compared to 1/12 (8.3%) from Group B and none from Group C.

Discussion

HIV infection in the pediatric population in India is virtually unknown and only anecdotal reports exist(1). AIDS has not been reported in any child from this country so far. Besides perinatally transmitted infection, infection through blood/blood products is the most likely route of infection for any pediatric population, and thus transfusion dependent children are at a high risk of contracting HIV infection. Since donor screening has been in effect in Delhi only for the last 3.5 years, all transfusion dependent children over 4 years of age have received unscreened blood.

The sero-positivity in donated blood in India is estimated to be between 0.1-1.5%(1,2). Outbreaks of seropositivity among paid blood donors (seropositivity as high as 75%) has been reported(9). Indirect evidence of high HIV seropositivity among commercial blood donors comes from a study by Tripathi *et al.*(9) which reported 17.6% seropositivity in indigenously manufactured blood products.

There is limited data available on the HIV status of blood/blood product recipients in India, and in a few reports available, between 4.4-12.1% of hemophelics have been shown to be seropositive but surprisingly the incidence among thalassemics is negligible(10,11). The reasons for such a high rate of seropositivity (8.9%) in our study is speculative, since most of the blood these children received were from voluntary donors. An outbreak of seropositivity among the donors, as has been reported from Pune(8) is a distinct possibility. Professional blood donors masquerading as voluntary donors and donating blood at different blood banks in different cities is also known(12), and could explain the HIV infection in these children.

The clinical features of the children with AIDS in our study were similar to those described previously(13). Weight loss, prolonged pyrexia and generalized lymphadenopathy were the most consistent features. Epistaxis, extensive purpuric skin lesions and thrombocytopenia, which were seen in half of these children, is generally not well described in literature. Alopecia, which was seen in 2/6 children, is another feature not commonly described in pediatric AIDS.

Recipients of multiple transfusions are known to have abnormalities in their immune status as reflected by alterations in the number and proportions of T-cell subsets with decrease in CD4/CD8 ratios and hypergammaglobinemia and depressed DCH(14). We, therefore, tested the immunologic functions of controls (HIV negative multi-transfused thalassemics), and compared them to the HIV positive asymptomatic children and with those with AIDS. Our study showed that DCH, CD4+ cell counts and CD4/CD8 ratios were good indicators of immunodeficiency. Absolute lymphopenia was a relatively uncommon feature, but it is known that fewer children than adults with AIDS have lymphopenia(15). Abnormalities of individual tests were found in all the groups in varying frequencies, and we thus found that a "battery" of tests gave a better assessment of the immunologic status. When the criteria of 2 abnormal results of the 4 tests done was applied to identify immunodeficiency, a very good correlation was found with the WHO clinical criteria – all the 6 children diagnosed to have AIDS could be designated as immunodeficient by these criteria.

Conversely, only 1/12 HIV positive asymptomatic and none of the controls were immunodeficient, though abnormalities in individual tests could be seen in a few.

Elevated levels of immunoglobulins are a feature of HIV infection in children(16). This finding has been singularly absent in out study. We are unable to offer an adequate explanation for this observation.

In conclusion it may be stated HIV infection and AIDS among multi-transfused children in India may not be as uncommon as previously thought(10,11). Though this is the first group of children identified, it is likely that such pools exist in other parts of the country as well, and a systematic search for them is warranted.

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