Original Article

Haematological Indices of HIV Infected Antiretroviral-naïve Children in Port Harcourt, Nigeria

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Afr J Haematol Oncol 2015;5:21-26

ABSTRACT

AIM Even though haematological abnormalities are common manifestations of HIV infection, few studies on haematological parameters in HIV-infected children have been carried out in sub-Saharan Africa. The aim of this study was to assess the haematological parameters in HIV-infected antiretroviral-naïve children attending the Infectious Disease Clinic of the University of Port Harcourt Teaching Hospital.

METHODS A prospective cross-sectional study which was carried out in children aged 5 -16 years attending the Infectious Disease Clinic of University of Port Harcourt Teaching Hospital over a two year period. The control population was HIV negative age and sex matched children. Haematological indices and CD4+ count were done at first visit as part of our routine workup. Patients were classified based on severity of their disease state in accordance with the WHO Clinical Staging of HIV/AIDS. The haematologic indices were estimated by automation and the CD4+ T lymphocytes were counted by flow cytometre. Antibodies to HIV were confirmed by two positive Rapid Diagnostic Test.A P value less than 0.05 was considered significant.

RESULTS One hundred and seven HIV infected antiretroviral naïve children were seen during this period. There were more females 66 (61.7%) than males 41 (38.3%). Most 52 (48.6%) of the mothers had secondary level of education while 35 (32.7%) and 20 (18.7%) had primary and tertiary levels of education respectively. The mean age was 7.4±1.34 years (range, 5-16 years). Haematological indices showed haematocrit of 26.98±0.70 (P=0.004); lymphocyte of 57.90±56 (P=0.13) and platelet of 86.95±16.59 (P=0.01). Most 79 (73.8%) of patients had moderate to severe immunosuppression. The mean CD4 count was 239.34±22.21 (P=0.01).

CONCLUSION This study has demonstrated that most children with HIV infection presented with significant anaemia, thrombocytopenia and moderate to severe immunosuppression.

Keywords: Haematological parameters; HIV; Children; Sub-Saharan Africa; Acquired Immunodeficiency Syndrome.

INTRODUCTION

Acquired Immune Deficiency Syndrome (AIDS) is a multi-system disease caused by HIV, and characterised by severe impairment and progressive damage of both cellular and humoral immune responses. In addition to CD4 lymphocyte cells, HIV replicates in dendritic cells and macrophages,[1-3] this replication disables the immune system and can lead to life threatening opportunistic infections. Apart from immunological abnormalities of HIV disease,[1] haematological complications have been shown as strong independent determinants of morbidity and mortality in HIV-infected individuals.[4]

Though numerous complications occur in HIV infected patients,[4-6] the most common haematological abnormalities are anaemia and neutropenia.[5]

These are mostly caused by inadequate blood cell production due to bone marrow suppression by HIV infection mediated by abnormal cytokine expression, alteration of the bone marrow microenvironment and as a complication of some of the antiretroviral agents.[7,8] Anaemia in many series have been found to occur in 20-70% of HIV infected patients and are associated with HIV disease progression and subsequent increased mortality.[7,9] The anaemia may be due to chronic infection, poor nutrition, autoimmune factors, virus-associated conditions (haemophagocytic syndrome, parvovirus B19 red cell aplasia), or the adverse effect of drugs (zidovudine).[9] Severe forms of anaemia in these individuals is associated with low CD4 levels and progression to AIDS[10] and is one of the strongest predictors of HIV mortality and poor responses to antiretroviral therapy (ART).[4]
Neutropenia is commonly found in advanced stages of HIV infection after development of AIDS, and has been associated with certain types of antiretroviral medications used in the treatment of HIV infection.[11] Thrombocytopenia is characterised by platelet counts below $125\times10^3/mm^3$, and also frequently occurs in HIV-infected patients.[12-14] Anaemia and leukopaenia in HIV-infected HAART-naïve patients result in poor ART-treatment outcome and otherwise strongly predict mortality.[4,15,16]

Although haematological complications are common features of HIV infection and AIDS, and may have a far reaching impact on patients' well-being, treatment and care, few studies on haematological parameters in HIV-infected children have been carried out in this sub-region. Such information for HIV-infected children in Port Harcourt may help to inform treatment and monitoring of HIV-infected individuals in this region. We therefore reviewed the haematological indices in HIV-infected HAART-naïve Children presenting to the University of Port Harcourt Teaching Hospital.

METHODS

This was a prospective cross-sectional study which was carried out in children aged 5 -16 years attending the Infectious Disease Clinic of University of Port Harcourt Teaching Hospital(UPTH) over a two year period (from January 2013 - December 2014). One hundred and seven HIV infected antiretroviral naïve children were seen during this period. Patient demographic data were documented (gender, age, parents' educational status and occupation). The control population was age and sex matched children who were HIV negative. Haematological indices and CD4+ count were done at first visit as part of our routine workup to assess the disease status and the need for antiretroviral therapy. Patients were classified based on severity of their disease state in accordance with the World Health Organization (WHO) Clinical Staging of HIV/AIDS.

The haematologic indices were estimated by automation and the CD4+ T lymphocytes were counted by flow cytometre. Antibodies to HIV were confirmed by two positive Rapid Diagnostic Tests (RDT). Erythrocyte sedimentation rate (ESR) measurement was carried out using Westergreen method as described by Dacie& Lewis.[17] Two ml of blood was diluted in 0.5 ml of trisodium citrate solution. The Westergreen pipette was filled to a zero mark and mounted on the Westergreen stand for one hour for the red cells to sediment with the aid of gravitational force. Then the column of the sedimented red cells was read at exactly 1 hour and results were recorded in mm/hr. Ethical clearance was obtained from the Ethics Committee of UPTH. Parents gave a written informed consent.

Statistical analyses were performed using SPSS v.18 (SPSS, Chicago, Illinois, USA). Student t-test where appropriate were used. A p-value less than 0.05 was considered significant.

RESULTS

One hundred and seven HIV infected antiretroviral naïve children were seen during this period. There were more females 66 (61.7%) than males 41 (38.3%). Demographic features (Table1) showed that most 52 (48.6%) of the mothers had secondary level of education while 35 (32.7%) and 20 (18.7%) had primary and tertiary levels of education respectively. The mean age was 7.4 ±1.34 years (range, 5-16 years). Haematological indices obtained when compared with age and sex matched controls are shown in Table 2. The mean haematocrit, leucocyte and platelet levels were significantly lower than the control. The mean

Table 1. Demographic and Baseline Characteristics of Antiretroviral-naïve Children at Presentation

Variables	Number	Percentage	Control (%)
Gender			
Male	41	39.3	41(39.3)
Female	66	61.7	66(61.7)
Age(years)			
5-<10	86	80.4	86(80.4)
10-16	21	19.6	21(19.6)
Mothers Educational Status			
Primary	35	32.7	23(21.5)
Secondary	52	48.6	35(32.7)
Tertiary	20	18.7	49(45.8)
Clinical Staging			
1-2	37	34.6	
3-4	70	65.4	
CD4+Cell count(cells/mm^3)			
<200	46	43.0	
200-499	33	30.8	
>500	28	26.2	

Table 2. The Haematologal and CD4+ Cell Count Findings of HIV naïve Children at the Time of Diagnosis

Laboratory Features	Naïve Patients	Controls	t	P value
Haematocrit(%)	26.98±0.70	30.41 ±0.97	2.931	0.004
White blood cell(g/l)	9.36±0.70	13.14±0.86	3.463	0.001
Lymphocyte (%)	57.90±56	51.68±2.15	1.537	0.13
Neutrophils (%)	40.462±3.33	46.53±2.61	1.437	0.15
Monocyte(%)	0.63±0.24	0.27±0.10	1.599	0.11
Eosinophil(%)	0.61±0.18	0.42±0.10	0.957	0.34
Basophil(%)	0.44±0.25	0.00	2.261	0.03
Platelet(g/l)	86.95±16.59	155.75±14.67	2.575	0.01
Erythrocyte sedimentation rate	65.68±8.71	76.36±6.15	1.021	031
CD4+cell count	239.34±22.21	415.61±29.61	4.249	0.01

erythrocyte sedimentation rate (ESR) was higher among controls (p=0.31). The WHO Clinical Staging System showed that 70 (65.4%) symptomatic patients were in advanced disease stages (3 and 4) while 37 (34.6%) patients were in stages 1 and 2. Most (79, 73.8%) of the patients had moderate to severe immunosuppression (Table 2). The mean CD4 count was 239.34±22.21 (P=0.01).

DISCUSSION

HIV infection is known to cause varied degrees of immuno-depression in man and this has massive haematologic concerns. The mean haematocrit was significantly lower than the control in this study. Anaemia is a very common finding in patients with HIV infection, particularly in individuals with more advanced HIV disease. In a study of patients receiving no myelosuppressive therapies, 8% of asymptomatic HIV-seropositive patients, 20% of those with symptomatic middle-stage HIV disease, and 71% of those with Centers for Disease Control (CDC)-defined AIDS were anaemic.[18] Also a study of serum immunoreactive erythropoietin in HIV-infected patients in various stages of illness showed that levels of the hormone failed to rise commensurately with increasing anemia, suggesting that insufficient amounts of erythropoietin may be one cause of anaemia in this setting.[19] Other studies have suggested that soluble factors in the serum of HIV-infected patients may inhibit haematopoiesis, or that direct HIV infection of marrow progenitor cells may play a role in producing anaemia and other haematologic abnormalities associated with HIV infection.[20] Infection with *Mycobacterium avium* complex (MAC) is another common cause of anaemia in advanced HIV disease.[21] Zidovudine (AZT) therapy is probably the most common cause of anaemia in HIV-infected patients. In the original phase II clinical trial that demonstrated the efficacy of AZT in patients with advanced HIV disease, statistically significant reductions in haemoglobin levels occurred in 34% of subjects receiving AZT (1,200 mg per day) following 6 weeks of therapy.[22]Antierythrocyte antibodies produce a positive direct antiglobulin test in approximately 20% of HIV-infected patients with hypergammaglobulinemia.[23]

Association of HIV infection with thrombocytopenia was long ago recognized.[24] References of a few cases and their description in the literature

indicate that patients might have AIDS rather than simple HIV infection, though more speculations would be unscientific. We observed that the mean platelet count was significantly reduced ($P < 0.05$), when compared with the controls. According to Sullivan, [25] it may be as a result of increased platelet destruction or decreased platelet production in subjects not on ART. This may tend to affect the normal haemostasis such that the individual becomes predisposed to bleeding tendency.

We found slightly elevated mean lymphocyte count in our patients when compared with the control ($P=0.13$). In HIV infection, an elevated lymphocyte count is not commonly part of the natural history of the infection. When it is found, efforts should be undertaken to discover its etiology and clinical consequences in a specific way. The most common finding in HIV infection over time is actually lymphopenia. A recent review attributed this to different phenomena over the natural history of the infection.[26] Early in HIV infection, there is viral destruction of selected memory T-cell populations, followed by a combination of profound increases in overall memory T-cell turnover, damage to the thymus and other lymphoid tissues, and ultimately, physiologic limitations in peripheral T-cell renewal. If lymphocytosis is evident, there should be concern regarding concomitant HTLV-1 infection, which is often seen in certain groups in specific geographic areas.[27]

The CD4 count is an indicator of immune status and stage of HIV infection. Most (79, 73.8%) of the children in the study group presented with moderate to severe degrees of immunosuppression as their CD4 counts were significantly less 500 cells/mm^3. This is in keeping with other studies worldwide. [5,28,29] The hallmark of HIV-1 infection is progressive depletion of the CD4 helper-inducer subset of lymphocytes. The underlying disorder affects the patient's cell-mediated immunity, resulting in absolute lymphopenia and reduced subpopulations of helper T lymphocytes (CD4+). Moreover, before a complete clinical manifestation of the disease occurs, its prodrome, "pre-AIDS", is frequently characterized by unexplained chronic lymphadenopathy (swollen lymph glands which likely indicate hyperstimulated antibody mediated immunity] or leukopenia involving helper T lymphocytes [low CD4 counts). This leads to the severe immune deficiency of the patient and suggests that a specific subset of T-cells could be a primary target for an infectious agent.[30]

Varying degrees of leucopenia has been reported in children with HAART-naïve HIV infection and often neutropenia occurs.[9,31,32] The mean leucocyte count in our study was within normal limits but varied significantly ($P<0.05$) with that of the control population. Circulating antineutrophil antibodies have been implicated in some cases, also multiple drugs used for treatment or prophylaxis for opportunistic infections may be incriminated. Though our patients were HAART-naïve some were on prophylaxis for *Pneumocystis jiroveci* pneumonia and this may have contributed to the low levels of leucocytes observed in our study.
Neutropenia, as observed in our report, is commonly reported among HIV infected individual in sub-Saharan Africa. [33-35] This may be due to HIV suppression of bone marrow resulting in abnormal granulopoesis. And anti-granulocyte antibodies have been described in HIV-infected persons.[36]

CONCLUSION

Haematologic manifestations are common in HIV-infected patients. Significant numbers of patients show anaemia, leucopenia and thrombocytopenia. This presupposes that patients with HIV infection should be investigated and treated for haematological abnormalities to reduce the morbidity of the patient.

REFERENCES

1. Rudnicka D, Schwartz O. Intrusive HIV-1-infected cells. Nat Immunol 2009;10:933–4.
2. Steinman RM, Granelli-Piperno A, Pope M, et al. The interaction of immunodeficiency viruses with dendritic cells. Curr Top MicrobiolImmunol 2003;276:1–30.
3. Lekkerkerker AN, van Kooyk Y, Geijtenbeek TB. Viral piracy: HIV-1 targets dendritic cells for transmission. Curr HIV Res 2006;4:169–76.
4. Anastos K, Shi Q, French A, Levine A, Greenblatt RM, Williams C et al. Total lymphocyte count,

hemoglobin and delayed-type hypersensitivity as predictors of death and AIDS illness in HIV-1 infected women receiving highly active antiretroviral therapy. J Acquir Immune DeficSyndr 2004;35:383–92.
5. Ajayi AO, Ajayi EA, Fasakin KA. CD4+ T-lymphocytes cell counts in adults with human immunodeficiency virus infection at the medical department of a tertiary health institution in Nigeria. Ann Afr Med 2009;8:257–60.
6. Coyle TE. Hematologic complications of human immunodeficiency virus infection and the acquired immunodeficiency syndrome. Med Clin North Am 1997;81:449–70.
7. Obirikorang C, Yeboah FA. Blood hemoglobin measurements as a predictive indicator for the progression of HIV/AIDS in resource-limited setting. J Biomed Sci 2009;16:102
8. Aboulafia DM, Mitsuyasu RT. Hematologic abnormalities in AIDS. HematolOncolClin North Am 1991;5:195.
9. Ram Y, Ellen GC. Acquired Immunodeficiency syndrome (Human immunodeficiency virus). In: Behrman RE, Kliegman RM, Jenson HB.(eds). Nelson textbook of pediatrics 18th Edition W.B. Saunders Company 2007; chapter 275 : 876-879
10. Mata-Marín JA, Gaytán-Martínez JE, Martínez-Martín RE, et al. Risk factors and correlates for anemia in HIV treatment-naïve infected patients: a cross-sectional analytical study. BMC Res Notes 2010;3:230
11. Levine A, Karim R, Mack W, Gravink DJ, Anastos K, Young M. et al. Neutropenia in human immunodeficiency virus infection: data from the Women's Interagency HIV Study. Arch Intern Med 2006;166:405 10.
12. Miguez-Burbano MJ, Jackson J Jr, Hadrigan S. Thrombocytopenia in HIV disease: clinical relevance, physiopathology and management. Curr Med Chem Cardiovasc Hematol Agents 2005;3:365–76.
13. Kirchhoff F, Silvestri G. Is Nef the elusive cause of HIV-associated hematopoietic dysfunction? J Clin Invest 2008;118:1622–5.
14. Dilkshit B, Wanchu A, Sachdeva RK, Sharma A, Das R. Profile of haematological abnormalities of Indian HIV infected individuals. BMC Blood Disorders 2009;9:5.
15. Siegfried N, Uthaman OA, Rutherford GW. Optimal time for initiation of antiretroviral therapy in asymptomatic, HIV-infected, treatment-naive adults. Cochrane Database Syst Rev 2010;(3): CD008272.
16. Wisaksana R, Sumantri R, Indrati AR, Alisjahbana B, Jusuf H, Sudjana P, et al. Anemia and iron homeostasis in a cohort of HIV-infected patients in Indonesia. BMC Infect Dis 2011;11:213.
17. Dacie JV, Lewis SM. Practical Haematology, 9th ed. Churchill Livingstone, London, 2001; 19-56.
18. Zon LI, Groopman JE. Hematologic manifestations of the human immune deficiency virus (HIV). SeminHematol 1988 Jul;25(3):208-18.
19. Spivak JL, Barnes DC, Fuchs E, Quinn TC. Serumimmunoreactive erythropoietin in HIV-infected patients. JAMA. 1989 Jun;261(21):3104-7
20. Stella CC, Ganser A, Hoelzer D. Defective in vitro growth of the hemopoietic progenitor cells in the acquired immunodeficiency syndrome. J Clin Invest. 1987 Aug;80(2):286-93
21. Hawkins CC, Gold JW, Whimbey E, Kiehn TE, Brannon P, Cammarata R, Brown AE, Armstrong D. Mycobacterium avium complex infections in patients with the acquired immunodeficiency syndrome. Ann Intern Med. 1986 Aug;105(2):184-8
22. Richman DD, Fischl MA, Grieco MH, Gottlieb MS, Volberding PA, Laskin OL, Leedom JM, Groopman JE, Mildvan D, Hirsch MS. The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex.A double-blind, placebo-controlled trial. N Engl J Med. 1987 Jul;317(4):192-7
23. Toy PT, Reid ME, Burns M. Positive direct antiglobulin test associated with hyperglobulinemia in acquired immunodeficiency syndrome (AIDS). Am J Hematol. 1985 Jun; 19(2):145-50
24. Sullivan PS, Hanson DL, Chu ST. Surveillance for thrombocytopenia in persons infected with HIV results from the multistate adult and adolescent spectrum of disease project J. Acquir. Immuno Deficiency Syndrome 1992;28:769-10
25. Sullivan PS, Hanson DL, Chu SY, Jones JL, Ciesielski CA. Surveillance for thrombocytopenia in persons infected with HIV: results from the multistate Adult and Adolescent Spectrum of Disease Project. J Acquir Immune DeficSyndr Hum Retrovirol. 1997;14:374-9.
26. Douek DC, Picker LJ, Koup RA. T cell dynamics in HIV-1 infection. Annu Rev Immunol. 2003;21:265-304.
27. Watanabe T. HTLV-1-associated diseases. Int J Hematol. 1997;66:257-278.
28. Ashton LJ, Carr A, Cunningham PH, Roggensack M, McLean K, Law M, Robertson M, Cooper DA, KaldorJM . Predictors of progression in long-term nonprogressors. Australian Long-Term Nonprogressor Study Group. AIDS Res Hum Retroviruses 1998;14(2):117-21
29. AtzoriC . In *Vitro* activity of HIV protease inhibitors against

Pneumocystis carinii. J Infect Dis 2000; 181:1629-1634.
30. Popovic M., Sarngadharan M.G., Read E. and Gallo R.C. Detection isolation and continuous production of cytopathic retroviruses (HTLV-111) from patients with AIDS and pre-AIDS. Science 1984; 224: 497-500.
31. Amballi AA, Ajibola A, Ogun SA: Demographic pattern and haematological profile in people living with HIV in a university teaching hospital. *Sci Res Essay 2007, 2(8):315-318.*
32. Erhabor O, Ejele OA, Nwauche CA, Buseri FI. Some haematological parameters in human immunodeficiency virus (HIV) infected Africans: the Nigerian perspective.
33. Mugisha JO, Shafer LA, Van der Paal L. Anaemia in a rural Ugandan HIV cohort: prevalence at enrolment, incidence, diagnosis and associated factors. Trop Med Int Health 2008;13:788–94.
34. Erhabor O, Ejele OA, Nwauche CA. Some hematological parameters in human immunodeficiency virus (HIV) infected Africans: the Nigerian perspective. Niger J Med 2005;14:33
35. Firnhaber C, Smeaton L, Saukila N. Comparisons of anemia, thrombocytopenia, and neutropenia at initiation of HIV antiretroviral therapy in Africa, Asia, and the Americas. Int J Infect Dis 2010;14:e1088–92.
36. Kimura S, Matsuda J, Ikematsu S. Efficacy of recombinant human granulocyte colony-stimulating factor on neutropenia in patients with AIDS. AIDS 1990;4:1251–5