Changes in the haematological parameters of HIV-1 infected children at 6 and 12 months of antiretroviral therapy in a large clinic cohort, North-Central Nigeria

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Abstract

Background: Prior to commencing antiretroviral therapy (ART), haematological abnormalities are a common occurrence in individuals diagnosed with human immunodeficiency virus (HIV). In the course of receiving ART, these abnormalities usually improve. We determined the prevalence of haematological abnormalities in children diagnosed with HIV-1 and the changes in haematological parameters that occur after 6 and 12 months of being on ART.

Methods: A cross-sectional study of HIV-1 infected children aged 2 months to 15 years, between July 2005 and March 2013, at the paediatric HIV clinic of the Jos University Teaching Hospital, Jos. Median values of repeated measures were compared using the Wilcoxon signed-rank sum test.

Results: The prevalence of anaemia, thrombocytopenia and leukopenia among the 941 children studied, prior to ART was 6.4%, 7.0% and 8.6%. Median (IQR) haemoglobin (Hb) levels increased from 10 g/dL (9–11 g/dL) at baseline to 11 g/dL (10–12 g/dL) and 11 g/dL (10–12 g/dL) at 6 and 12 months of ART (P < 0.001 and P < 0.001), respectively, a 10% increase in both cases. Also, platelet count increased from a median of 327 × 10^3/μL (243–426 × 10^3/μL) at baseline to 333 × 10^3/μL (266–408 × 10^3/μL) at 6 months and 339 × 10^3/μL (267–420 × 10^3/μL) at 12 months, representing a 1.8% and 3.7% increase, respectively. The median total white blood cell count decreased from 7.4 × 10^3/μL (5.3–9.9 × 10^3/μL) at baseline to 5.9 × 10^3/μL (4.6–8.0 × 10^3/μL) and 5.8 × 10^3/μL (4.5–7.5 × 10^3/μL) at 6 and 12 months of ART (P < 0.001 and P < 0.001), a 20.3% and 21.6% decrease, respectively.

Conclusion: During the 12 months of ART, children in our cohort had significant improvements in haematological parameters such as haemoglobin levels and platelet counts, which would suggest an early positive response to ART.

Keywords: haematological, parameters, HIV-1, ART, children

Introduction

Prior to commencing antiretroviral therapy (ART), haematological abnormalities are a common occurrence in individuals diagnosed with human immunodeficiency virus (HIV) [1–12]. The commonest of these abnormalities reported by several studies include: anaemia [1,4–6,10–12], leukopenia [3,4,7] and thrombocytopenia [3,12]. Anaemia is the most common haematological abnormality in HIV-infected children prior to starting ART [1,2,4]. A previously reported prevalence of anaemia in treatment-naïve children in Nigeria was 38.2% [2], while studies from Kenya and India reported a prevalence of 35.9% [13] and 69% [4], respectively. In the course of receiving ART, haematological abnormalities could also occur from adverse events secondary to ART [7,13–15], but in most cases, these abnormalities improve with ART [7,9,13,16].

The causes of, or associated risk factors for, the anaemia, leukopenia and thrombocytopenia both prior to starting ART and while on ART could include HIV-related bone-marrow suppression, myelosuppression from antiretroviral drugs such as zidovudine and opportunistic infections (Mycobacterium avium complex, parvovirus B19 and cytomegalovirus) [1,17,18]. Monitoring changes in haematological abnormalities in children while they are receiving ART could be valuable, as improvement in these abnormalities in the course of ART, could be a useful indicator of a good response to ART.

In this study we determined the prevalence of haematological abnormalities in children diagnosed with HIV and the changes in haematological parameters that occur after 6 and 12 months of being on ART among HIV-infected children attending a large paediatric HIV clinic in Jos, Nigeria.

Methods

This was a cross-sectional study that utilised data already captured in an electronic medical records system (EMRS) as part of an ongoing HIV/AIDS treatment, care and support programme. Children diagnosed with HIV and commenced on ART had their haematological parameters determined at 6 and 12 months of ART. Children aged from 2 months to 15 years and enrolled into HIV treatment and care July 2005–March 2013 at the paediatric HIV clinic of the Jos University Teaching Hospital were studied. This clinic provides HIV care services for the city of Jos and is the largest ART referral centre in North–Central Nigeria.

For this study a haemoglobin (Hb) level of <8 g/dL was used to define anaemia for children in all age groups; WHO define moderate anaemia as ranging from 7 g/dL to 10.9 g/dL for children aged 6 months–15 years [19]. Leukopenia, neutropenia and thrombocytopenia were defined as a total white blood cell (WBC) count <4.0 × 10^3 cells/μL, absolute neutrophil count (ANC) <1.0 × 10^3 cells/μL and total platelet count (TPC) <150 × 10^3/μL.
Lymphopenia was considered an absolute lymphocyte count (ALC) <0.8×10⁹ cells/μL [6].

Haematological parameters were measured using Mindray 3200 Auto Haematology Analyzer (Shenzhen Mindray Bio-Medical, Shenzhen, China). We have previously described in detail, the techniques used in diagnosing HIV in children as well as the measurement of viral load and CD4 cell count [20]. HIV diagnosis and the criteria for initiating ART in the children were based on the Nigerian National Guidelines for Pediatric HIV and AIDS Treatment and Care [21,22]. For most patients, ART was started within 2–4 weeks of HIV diagnosis. The most commonly used ART regimen was zidovudine (AZT) + lamivudine (3TC) + nevirapine (NVP); however, if the patient was diagnosed with pulmonary tuberculosis (PTB) at the time of ART initiation, NVP was replaced with efavirenz (EFV) in the regimen.

For statistical analysis, some continuous variables were dichotomised using defined cut-off values (as mentioned above), into haematological abnormalities (anaemia, leukopenia, neutropenia, thrombocytopenia and lymphopenia). The outcome measures were a comparison of the proportions of those with or without a haematological abnormality and a comparison of repeated measures of haematological values. A two-sample test of proportion for the difference in proportion was used to compare the proportion of those with an abnormality at baseline, to the proportion of those with that abnormality at 6 months and at 12 months of ART, respectively. Comparison of repeated measures was performed using the Wilcoxon signed-rank sum test for continuous variables that were not normally distributed. P-values were determined in all cases, with values <0.05 considered statistically significant. All analyses were performed using Stata software version 10.0 (Stata Corporation, College Station, Texas, USA).

Ethical approval for the use of secondary data was obtained from the Ethics Committee of the Jos University Teaching Hospital, AIDS Prevention Initiative in Nigeria (APIN) Ltd, Abuja and Harvard TH Chan School of Public Health, Boston, USA. Parents/guardians of the children had given a written informed consent.

**Results**

Of the 941 children studied, the majority were females (488, 51.9%), in the age group 6 months–15 years (596, 63.2%), and had a median age (IQR) of 3.6 years (1.7–6.8 years). There were 487 (55.7%) children in WHO clinical stage 1 and 2 and 387 (44.3%) with severe immunosuppression at enrolment into care. Their baseline median Hb levels, platelet counts, total WBC counts and total lymphocyte counts were all within normal ranges; but 58 (6.4%), 63 (7%), 78 (8.6%) and 13 (1.4%) had anaemia, thrombocytopenia, leukopenia and lymphopenia, respectively (Table 1).

The median (IQR) Hb levels increased from 10 g/dL (9–11 g/dL) at baseline to 11 g/dL (10–12 g/dL) at 6 months of ART and remained at 11 g/dL (10–12 g/dL) at 12 months of ART, respectively (P<0.001 and P<0.001), a 10% increase in both cases. Also, the proportion of children with anaemia reduced from 6.4% before commencement of ART to 2.3% at 6 months of ART, a 64% reduction (P<0.001) and the proportion of those with anaemia reduced from 6.4% at baseline to 1.3% by 12 months of ART, an 80% reduction (P<0.001) (Table 2).

| Table 1. Baseline characteristics of HIV-1-infected children receiving antiretroviral therapy |
|--------------------------------------------------|----------------------------------|
| **Characteristics**                              | **Total**                        |
| Age (years)                                      | 345 (36.7)                      |
| 0–5                                              | 356 (38.1)                      |
| 6–15                                             | 596 (63.3)                      |
| Median (IQR)                                     | 3.6 (1.7–6.8)                   |
| Sex                                              | 453 (48.1)                      |
| Male                                             | 488 (51.9)                      |
| Female                                           | 81 (8.8)                        |
| WHO clinical stage                               |                                  |
| Stage 1 and 2                                    | 487 (55.7)                      |
| Stage 3 and 4                                    | 387 (44.3)                      |
| Stage 1–3                                        |                                  |
| Severe immunosuppression                          |                                  |
| Present                                          | 369 (42.9)                      |
| Absent                                           | 492 (57.1)                      |
| CD4 cell count (cells/μL)                        | 508 (252–842)                   |
| CD4 (%)                                          | 17 (10–26)                      |
| Viral load (copies/mL)                           | 444,480 (6638–202,186)          |
| Log viral load (copies/mL)                       | 10.7 (8.8–12.2)                 |
| Haemoglobin level (g/dL)                         | 10 (9–11)                       |
| Platelet count (>10¹¹/μL)                        | 327 (243–426)                   |
| Total WBC count (>10¹⁰/μL)                       | 7.4 (5.3–9.9)                   |
| Total lymphocyte count (>10⁹/μL)                 | 3.6 (2.4–5.3)                   |
| Total neutrophil count (>10⁹/μL)                 | 2.4 (1.7–3.7)                   |
| Anaemia                                          |                                  |
| Present                                          | 58 (6.4)                        |
| Absent                                           | 847 (93.6)                      |
| Thrombocytopenia                                 |                                  |
| Present                                          | 63 (7.0)                        |
| Absent                                           | 838 (93.0)                      |
| Leukopenia                                       |                                  |
| Present                                          | 78 (8.6)                        |
| Absent                                           | 827 (91.4)                      |
| Lymphopenia                                      |                                  |
| Present                                          | 13 (1.4)                        |
| Absent                                           | 891 (98.6)                      |
| Neutropenia                                      |                                  |
| Present                                          | 45 (5.0)                        |
| Absent                                           | 853 (95.0)                      |

Data are presented as number (%) or median (interquartile range (IQR)). BMI: body mass index; WBC: white blood cells. ** HIV severe immunodeficiency based on World Health Organization (WHO) classification.

The median (IQR) platelet count increased from 327×10³/μL (243–426×10³/μL) at baseline, to 333×10³/μL (266–406×10³/μL) at 6 months and 339×10³/μL (267–420×10³/μL) at 12 months of ART, a 1.8% and 3.7% increase, respectively. The proportion of children with thrombocytopenia significantly reduced from 7.0%
at baseline to 4.1% at 6 months and 3.6% at 12 months of ART, respectively (P<0.016 and P<0.005), a 41.4% and 48.6% decrease in both cases (Table 2).

The median (IQR) total WBC decreased from 7.4×10^3/μL (5.3–9.9×10^3/μL) at baseline to 5.9×10^3/μL (4.6–8.0×10^3/μL) and 5.8×10^3/μL (4.5–7.5×10^3/μL) at 6 months and 12 months of ART, respectively (P<0.001 and P<0.001), a 20.3% and 21.6% decrease; and the proportion of children with leukopenia increased significantly from 8.6% at baseline to 15.2% at 6 months and 15.1% at 12 months of ART, respectively (P<0.001 and P<0.001) (Table 2).

The median (IQR) absolute lymphocyte count increased to 15.1% at 12 months of ART, respectively (P<0.001 from 8.6% at baseline to 4.1% at 6 months and 3.6% at 12 months of ART, and the proportion of children with leukopenia increased from 98.6% at baseline to 73.7% at 6 months of ART and 67.3% at 12 months of ART, respectively (P<0.001 and P<0.001) (Table 2).

**Discussion**

Prior to ART, the prevalence of anaemia, thrombocytopenia and leukopenia in this cohort of children was 6.4% (58/905), 7.0% (63/901) and 8.6% (78/905), respectively. With ART, the median Hb level and platelet counts improved while higher but normal total WBC count levels declined to lower normal levels.

The prevalence of anaemia in our study was low (6.4%) compared to previous studies in ART-naive children in Nigeria (38.2% [2]), Ethiopia (21.9% [14]), Kenya (35.9%[13]) and India (69% [4]) but slightly higher than one Nigerian study (3% [8]). In all these studies, AZT was part of all the ART combinations used. The lower prevalence of anaemia in our study may partly be attributed to the lower proportion (14.7%) of children with severe malnutrition (BMI z-score ≤−3) compared to a higher proportion (35.9%) of children with severe malnutrition (HFA z-score ≤−3) in the Kenyan study. It has been shown that HIV-infected children with malnutrition have an increased risk for anaemia [23].

The improvement (increase) in the Hb levels while on ART seen in our study was similar to those reported by several studies both in children [13,14] and adults [9,16,24]. In one of these studies, there was a 6.7% increase in Hb levels after 6 months of ART with a significant decrease in the proportion of children with anaemia from 6.4% before ART to 2.3% after ART, in our study. In another similarly large paediatric cohort in Zambia [25], a 9.7% increase in Hb levels was observed after 6 months of ART, similar to the 10% increase observed in our study. However in other studies, the Hb levels in individuals receiving ART decreased and some even developed anaemia, usually attributed to the adverse effects of AZT [20,26].

An improvement in platelet counts as well as a decrease in the proportion of children with thrombocytopenia while on ART seen in our study is consistent with those of several studies [7,13,14]. In the study of Kenyan children [13], the median platelet count before and after 6 months of ART was 255×10^3/μL and 279×10^3/μL, a 9.4% increase. Our study showed a similar trend with a count

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**Table 2. Comparison of haematological parameters at baseline, 6 months and 12 months of antiretroviral therapy**

| Parameter                  | At baseline | At 6 months of ART | P value | At 12 months of ART | P value |
|----------------------------|-------------|--------------------|---------|--------------------|---------|
| Haemoglobin level (g/dL)   | Median (IQR)| 10 (9–11)          | <0.001  | 11 (10–12)         | <0.001  |
| Platelet count (×10^9/μL)  | Median (IQR)| 327 (243–426)      | 0.050   | 333 (266–408)      | 0.198   |
| Total WBC count (×10^4 cells/μL) | Mean (IQR)| 7.4 (5.3–9.9)      | <0.001  | 5.9 (4.6–8.0)      | <0.001  |
| Total lymphocyte count (×10^3 cells/μL) | Median (IQR)| 3.6 (2.4–5.3)      | <0.001  | 2.6 (0–4.0)        | <0.001  |
| Total Neutrophil count (×10^3 cells/μL) | Median (IQR)| 2.4 (1.7–3.7)      | <0.001  | 1.8 (1.2–2.7)      | <0.001  |
| Anaemia, n (%)             | Absent      | 847 (93.6)         | 0.016   | 682 (97.7)         | 0.005   |
|                           | Present     | 58 (6.4)           | <0.001  | 16 (2.3)           | <0.001  |
| Leukopenia, n (%)          | Absent      | 838 (93.0)         | <0.001  | 669 (95.9)         | <0.001  |
|                           | Present     | 78 (8.6)           | 0.001   | 106 (15.2)         | 0.001   |
| Lymphopenia, n (%)         | Absent      | 827 (91.4)         | <0.001  | 592 (84.8)         | <0.001  |
|                           | Present     | 13 (1.4)           | 0.001   | 248 (32.7)         | 0.001   |
| Neutropenia, n (%)         | Absent      | 891 (98.6)         | 0.001   | 693 (73.7)         | 0.001   |
|                           | Present     | 45 (5.0)           | 0.001   | 87 (12.5)          | 0.001   |

The Wilcoxon signed-rank sum test was used for comparison of two medians, while the two-sample test of proportion was used to compare the difference in the proportion of those with or without a haematological abnormality at baseline versus 6 months and at baseline versus 12 months. IQR: interquartile range; WBC: white blood cells; ART: antiretroviral therapy.
of 327×10³/μL at baseline and 333×10³/μL at 6 months of ART, a 1.8% increase. Additionally, in the Kenyan study the proportion of children with thrombocytopenia reduced from 20% to 6.5% after 6 months of ART, a 67.5% reduction. Our study showed a similar trend with a reduction of 41.4% from 20% to 6.5% after 6 months of ART.

In our study the median total WBC decreased from 7.4×10³/μL at baseline to 5.9×10³/μL and 5.8×10³/μL at 6 and 12 months of ART, a 20.3% and 21.6% decrease, respectively. This finding is similar to a Kenyan study in children where the median total WBC count at baseline was 9.4×10³/μL but decreased to 6.7×10³/μL by 6 months of ART, a 28.7% decrease [13]. A possible explanation for these findings of a decreased WBC count may be that at the time of enrolment into care, children may have been having intercurrent acute or chronic infections causing leukocytosis but following antibiotic treatment and ART the WBC levels decreased. This decrease could have been due to the antiretroviral drugs improving immunity, leading to fewer infections. Our study findings contrast to one study that reported a significant increase in WBC following ART (mean increase of 0.8×10³/μL) [27].

The overall improvement in the haematological abnormalities seen in this and other studies has been attributed to the positive effect of ART in the reduction of viral load, decreased destruction of mature haematopoietic cells of multiple lineages and an improvement in the blunted erythropoietin response [28], in addition to decreased incidences of opportunistic infections while on ART [27].

Our study is limited by its cross-sectional study design, which precludes firm conclusions that improvements in haematological parameters were mainly due to ART.

Conclusion

Improvements in haematological parameters such as haemoglobin levels and platelet counts occurred in our cohort of children while on ART, which would suggest an early positive response to ART.

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