Impact of First Line Antiretroviral Therapy on Clinical Outcomes Among HIV-1 Infected Adults Attending One of the Largest HIV Care and Treatment Program in Nairobi Kenya

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Abstract

Objective: This study evaluated the immunologic (CD4 cell count), virological (HIV RNA viral load), hepatic (alanine and aspartate aminotransferase - ALT and AST), renal (creatinine) and hematological (hemoglobin -HB, White Blood Cell - WBC, Lymphocytes - LYM and platelets - PLT) response to a six-months ART treatment among HIV participants in Nairobi Kenya.

Methods: Blood samples were obtained from 599 consenting HIV infected participants receiving HIV treatment in Nairobi. CD4 cell counts were measured using flow cytometer and viral load determined using real-time polymerase chain reaction. The blood hematology, liver and kidney function tests were also measured. One-way ANOVA and Linear regression analysis were conducted.

Results: The median age at ART initiation was 41 years (IQR 35-47 years). The majority of participants (60.3%) were female and 56.1% started on regimens with 2 NRTIs and efavirenz based NNRTI. About 40% of the participants were failing treatment 6 month post ART initiation. The CD4 count significantly increased at the 6-month post ART initiation (301.7 ± 199.4 to 329.4 ± 305.8; P<0.05). Hepatotoxicity (ALT and AST levels >5 times the upper limit of normal - ULN) and renal abnormalities (elevated serum creatinine levels) were all high at month 6 compared to baseline; ALT (2.5 to 10.5%), AST (5.3 to 23.4%) and creatinine (63.4 to 68.8%). Fewer participants at month 6 had anemia (29.4% verses 56.4%), leucopenia (42.4% vs. 46.9%) and thrombocytopenia (6.5% vs. 84.1%) compared to baseline. In multivariable models, baseline levels of this parameter, ART regimen and duration with HIV at ART initiation were the most important determinant of month 6 levels.

Conclusion: These data demonstrate sustained immunologic/virologic response to ART among participants remaining on therapy. Anemia, leucopenia and thrombocytopenia were minimized with marginal hepatotoxicity and renal impairment seen. Interventions leading to earlier HIV diagnosis and initiation of ART could substantially improve patient outcomes in Kenya.

Keywords: First line antiretroviral therapy; Clinical outcomes; HIV-1 infected adults; Largest HIV treatment program; Nairobi; Kenya

Introduction

Combination antiretroviral therapy (ART) use has slowed disease progression, decreased mortality and improved the quality of life for many persons with HIV [1-3]. The CD4 cell count and HIV RNA viral load are important measures of the efficacy and effectiveness of antiretroviral therapy (ART) among HIV participants enrolled in HIV care and treatment programs. Robust improvements in CD4 cell counts following ART initiation have been documented [2-4]. The CD4 count at ART initiation determines the degree of immunologic and virologic ART response [5,6] as well as subsequent risk of morbidity and mortality [1,7]. Increasingly however, adverse effects due to combination ART are being reported and are emerging as a major safety concern limiting the clinical benefits of these drugs. In particular, hepatotoxicity [8,9] and hematologic abnormalities [10], are common affecting the quality of life and are associated with HIV/AIDS progression and decreased survival.

Incidence of severe hepatotoxicity has been reported as 5 to 10 per 100 person-years during ART [11]. Almost every licensed ARV has been associated with liver enzyme elevations, with severe hepatic outcomes being frequent during treatment with NNRTI-based regimens than NRTI- or PI-based regimes [12,13]. Hematologic abnormalities such as peripheral blood cytopenia, anaemia, neutropenia, and thrombocytopenia have also been reported among HIV participants receiving ART [14].

As a buildup to reports documenting clinical and immunologic outcomes in sub-Saharan Africa [2] of ART comparable to those observed in resource-rich settings, we provide data on the immunological and virological responses as well as hepatotoxicity and hematological abnormalities due to ART among a cohort population on lifelong ART treatment in Nairobi Kenya.

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Methods
Study design and setting

This was a prospective observational cohort study. Data presenting in this study was part of a study that aimed at evaluating the etiology of sub-optimal responses to non-nucleoside reverse transcriptase inhibitor (NNRTI) among HIV participants receiving HIV care and treatment at the Family AIDS Care and Educational Services (FACES) based at Kenya Medical Research Institute (KEMRI) in Nairobi Kenya. FACES is a collaboration between the KEMRI and the University of California, San Francisco (UCSF), funded through the US President’s Emergency Plan for AIDS Relief (PEPFAR). The program is a family-focused, comprehensive HIV prevention, care, and treatment program that initially launched with one HIV site in Nairobi in September 2004 administered under CRDR-KEMRI. Cumulatively to date, this program has enrolled close to 4000 participants, nearly 90% being adults and 10% pediatrics. FACES program also offers counseling services, HIV testing and counseling, TB diagnosis, reproductive health, and nutritional support for the clinically malnourished and other laboratory services including CD4, blood chemistry, full blood count and liver function tests to improve the quality of care.

Sampling and enrollment

Using the population proportion estimation with specified relative precision sample size formula [15] and setting alpha (α) at 0.05, relative precision (e) at 0.07 and proportion of HIV-1 infected individuals experiencing virological failure with NNRTI sub-optimal plasma levels during a 6 month ARV was not expected to be below 60% [16]; a total of 599 participants were recruited to achieve 0.95 power.

Participants meeting the recruitment criteria (receiving first line ARV (Zidovudine (AZT) or Abacavir (ABC), 3TC, and EFV/NVP for six months, were consented and enrolled into the study using two sampling techniques; first, random sampling was used to intentionally enroll eligible participants. To increase the participants flow and shorten recruitment period the second technique the snowballing or word-of-mouth technique was used. In this case, the participants already enrolled were used as referral sources. These participants were given cards in order to recommend other eligible participants. Face to face interviews using structured questionnaires were used to collected both qualitative and quantitative data.

This study was carried out between 2014 and 2016 and was approved by Ethical Review Committee of Kenya Medical Research Institute (SSC No. 2539 on 21st May, 2013).

Data collection

A detailed structured interview was used to gathered information on demographic data, clinical history, adherence, stigma and medical history. The interviews were conducted six months after treatment initiation. Blood specimens were also collected for CD4 cell count, HIV RNA viral load, AST, ALT and full blood count testing. The blood samples were collected at the time of ART initiation and six-month post ART initiation.

Participants records

The medical files of recruited subjects were retrogressively retrieved and assessed for the following information: liver function tests, renal tests, CD4 count, and HIV RNA viral load and full blood count.

Hematological, blood chemistry, immunological and virological analyses

CD4 cell counts were measured using a FACSCount TM flow cytometer (BD Biosciences, San Jose, USA), which gives the results in absolute numbers and percentages. The plasma HIV-1 RNA were measured using Generic HIV Viral Load (Biocentric, Bandol, France), a real-time polymerase chain reaction assay based on long-terminal repeat with a detection limit of 300 RNA cp/ml in 0.2 ml of plasma.

The full blood cells were measured using the SYMEX KX21N hematology instrument (Sysmex Corporation, Kobe, Japan). Blood chemistry were conducted with a Lisa 300 Plus analyzer (Hycel Diagnostics, Massy, France).

Definition

Biochemical and hematological abnormalities were defined as follows: Liver hepatotoxicity was defined as ALT greater than 56 U/L or AST greater than 40 U/L. Renal abnormalities was considered when creatinine was greater than 0.8 mg/dL. Anemia was defined as hemoglobin <13 g/dl (men) and <12 g/dl (women) while leucopenia as total WBC count less than 4.3 × 10^9 cells per liter. Total platelet count <150 × 10^9/μl was considered as thrombocytopenia. Lymphocytosis, an increase in the number of lymphocytes was considered when the absolute lymphocyte count was greater than 4000 cells/μl.

Immunological failure was defined as CD4 count falls to the baseline (or below) or Persistent CD4 levels below 100 cells/mm. Virologic failure was defined as plasma viral load above 1000 copies/ml based after 6 months of treatment, with adherence support.

Statistical analysis

All data analysis was performed using SPSS version 21 package. Data was presented as means ± standard deviation (SD) and calculations carried out using the Student’s t-test and one-way ANOVA. Linear regression models were used to assess associations between predictor variables and laboratory outcomes following ART initiation. The level of significance was set at p<0.05 and confidence level at 95%.

Results

Month 6 characteristics of study participants

A total of 599 HIV infected participants on first line ARV d4T or AZT, 3TC and EFV/NVP were enrolled and their month 6 characteristics are summarized in Table 1. There were 357 (59.6%) participants responding versus 242 (40.4%) failing treatment. Among the 242 failures 21/242 (8.7%) had virologic failure, 14/242 (5.8%) with immunologic failure and 16/242 (6.6%) had combined virologic and immunologic failure while 207/242 (85.5%) had immunologic failures.

The majority (60.3%) of participants were female. Amongst these, 60.8% and 59.5% in the overall population were responders and in the failing cases respectively. The median age at ART initiation was 41 years (IQR 35–47 years). The ages of participants were generally similar between responding and failing participants (P=0.862). More than 64% of the participants were Bantus with no significant difference between responders and failures (P=0.524). The median duration with HIV disease for all participants was 6 years (IQR 4–8 years). Responders had HIV infection longer than the failures (P=0.017).

The overall median baseline CD4 cell count was 288 cells/ml (IQR 138–410 cells/ml). The responders had higher median CD4 cell count than failures (305 cells/ml [IQR 210–409 cells/ml] versus 206...
cells/ml [IQR 94-419 cells/ml]) (P=0.001). The overall mean VL was 2644.7 (range 1-367728) copies/ml. The failures had higher mean VL than responders (6484 copies/ml range 1-367728 copies/ml versus 418 copies/ml range 1-160 copies/ml) (P=0.001). The overall median baseline ALT level was 16.9 U/L (IQR 11-23.9 U/L) with no significant difference between responders and failures (P=0.583). The overall median 6 month hemoglobin (HB) level was 12.6 g/dl (IQR 11-14.3 g/dl) with no significant difference between responders and failures (P=0.065). There was no significant difference between responders and failures in the median WBC level (P=0.868), PLT (P=0.98) and LYMP (P=0.791) (Table 1).

Changes in the clinical outcomes 6 month post ART initiation

Immunological, hematological and biochemical changes 6
months post ART initiation is summarized in Table 2. The CD4 count significantly increased at the month 6 post ART (301.7 ± 199.4 to 329.4 ± 305.8; p<0.05). The levels of AST (19.5 ± 17.2 to 23.4; p<0.05) was significantly increased (upper range) at the 6 month post ART. There was no significant increase in the mean ALT levels 6 months into ART; ALT (20.8 ± 17.2 to 31 ± 20.5 U/L; p=0.495). Serum creatinine levels (0.97 ± 0.55 to 0.95 ± 0.47; P=0.095) decreased non-significantly from baseline to month 6 post ART initiation.

For all the participants, a consistent increase was noted in the hematological parameters from baseline to month 6 post ART initiation. Changes in the levels of laboratory parameters at baseline and month 6 among HIV infected participants undergoing ART treatment.

### Table 2: Changes in the levels of laboratory parameters at baseline and month 6 among HIV infected participants undergoing ART treatment.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean (± SD)</th>
<th>No</th>
<th>Baseline</th>
<th>Month 12</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ (cell/μL) Mean (± SD)</td>
<td>All</td>
<td>599</td>
<td>301.7 ± 199.4</td>
<td>329.4 ± 305.8</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Responders</td>
<td>357</td>
<td>329.4 ± 185.6</td>
<td>468.8 ± 312.2</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Failure</td>
<td>242</td>
<td>261.2 ± 212.3</td>
<td>123.6 ± 129.8</td>
<td>0.028</td>
</tr>
</tbody>
</table>

Laboratory data are presented using mean ± standard deviation. Month 6 data were compared against the baseline values using ANOVA at a 5% level of significance. Data with P>0.05 indicates significant difference.

### Prevalence of hepatotoxicity, nephrotoxicity and blood abnormalities

Various biochemical and hematological abnormalities that could have been associated with administration of antiretroviral drugs were observed (Figure 1). Hepatotoxicity due to elevated ALT and AST were much higher at month 6 compared to baseline; ALT (2.5% to 10.5%) and AST (5.3% to 23.4%). Participants with renal abnormalities due to elevated serum creatinine levels were higher at month 6 (68.8%) compared to baseline (63.4%). The study revealed higher cases of anemia (56.4%), leucopenia (46.9%) and thrombocytopenia (84.1%) among all the HIV infected participants at baseline. Conversely, these decreased (anemia 29.4%, leucopenia 42.4% and thrombocytopenia to 6.5%) at month 6 post ART initiation. Lymphocytosis (2.5%) was low at the first visit but showed higher values at 5.3% at month 6.

### Factors associated with clinical outcomes 6 month post ART

The independent variables associated with laboratory outcomes in multivariable regression models are summarized in Table 3. The CD4 cell counts (beta 0.441 cells/μL, 95% CI, 0.308 to 0.574 cells/μL, P=0.001) and duration with HIV disease (years) (beta 10.1 cells/μL, 95% CI, 1.707 to 18.484 cells/μL, P=0.018) at baseline significantly predicted month 6 CD4 cell count. Baseline AST (beta 0.711 U/L, 95% CI, 1.707 to 18.484 cells/μL, P=0.018) at baseline significantly predicted month 6 CD4 cell count. Baseline AST (beta 0.711 U/L, 95%
CI, 0.558 to 0.864 U/L; P=0.001) significantly predicted month 6 AST levels. ART regimen predicted the month 6 creatinine level (beta 0.088 mg/dL, 95% CI, 0.818 to 0.885 10⁻³ mm³; P=0.048).

In other models, baseline levels played a key role in the changes in the month 6 outcomes as shown in Table 3. None of the independent variables (ART regimen, duration of ART, duration with HIV disease, adherence, gender, age, baseline CD4, Log10-transformed viral load, baseline ALT) were predictors of month 6 ALT levels.

### Discussion

This study provides data on ART treatment outcomes among one of the largest cohort of HIV positive population receiving HIV care and treatment in Nairobi Kenya. The study demonstrates specific robust CD4 and HIV RNA viral load responses to ART treatment. The ART adverse reactions particularly hepatotoxicity and renal abnormalities were sustained at month 6 compared to baseline. Cases of anemia, leucopenia and lymphocytosis were observed. Other studies associated conditions [31]. In this study, cases of thrombocytopenia, malignancies and consequence of therapies used for HIV infection and underlying diseases, and concomitant drugs [29]. It is therefore generally recommended that long term effectiveness of ART particularly in HIV infected individuals who are able to adhere and remain on ART for extended periods.

Studies in resource-limited settings in Africa, Latin America, and Asia [17-19] demonstrate robust CD4 responses to ART that are sustained over several years. In this study, at month 6 the mean CD4 had increased by 27.7 cell/µL above that of baseline with some 43.7% of the participants having CD4 cell count above 350 cell/µL. Further, only 5.7% of the participants had HIV RNA viral load >1000 copies/mL while 94.3% attained viral suppression 6-month post ART treatment. This study reaffirms the positive virological and immunological response of the Largest HIV Care and Treatment Program in Nairobi Kenya. The study demonstrates specific robust CD4 cell count and HIV RNA viral load responses to ART treatment. The ART adverse reactions particularly hepatotoxicity and renal abnormalities were sustained at month 6 compared to baseline. Cases of anemia, leucopenia and lymphocytopenia dropped at the 6th month of ART treatment. Our results are thus encouraging and are a pointer to a long-term effectiveness of ART particularly in HIV Infected individuals who are able to adhere and remain on ART for extended periods.

This study showed that apart from the duration with HIV infection, patient's baseline CD4 remained the single most important factor determining CD4 count in month 6. This observation has been documented by other investigators [17,22] that participants with higher CD4 cell counts at ART initiation achieve a higher CD4 cell count in the following months and years. The importance of this observation cannot therefore be overstated. The baseline CD4 cell count, second only to subsequent medication adherence, is the most important predictor of clinical progression and survival after ART initiation [23,24].

Due to the increased ART scale up programs, increased focus on the toxicities and adverse reactions of combination ART, such as drug-induced liver injury, neuropathy, and pancreatitis continue to attract attention. Several studies show that liver injuries are the most common non-AIDS cause of death among people with HIV infection [25]. Further, during this era of effective ART, about 18% of deaths among HIV participants are due to liver-related complications [26,27]. Our study showed a significant elevation in the mean liver transaminase enzymes (AST and ALT) from baseline to month 6. Cases of hepatotoxicity due to elevated ALT and AST (>5 times ULN) were much higher at month 6 compared to baseline at 2.5% to 10.5% and 5.3% to 23.4%, respectively. Other clinical studies have indicated that grade 3 (ALT and/or AST levels >5 times the ULN) and grade 4 (ALT and/or AST levels >10 times the ULN) hepatotoxicity is observed in 5%–10% of HIV-positive participants treated with combination ART for >6 months [14,28].

Baseline hepatic parameters (AST and ALT) were great pointers to the month 6 levels. Studies have shown that HIV patient's susceptibility to the hepatotoxic effects of ART is due to the interplay of the effects of the ART and the associated risk factors, such as alcohol use, underlying diseases, and concomitant drugs [29]. It is therefore generally recommended that long term administration of ART should be carefully monitored to avoid possible drug induced injuries.

Creatinine level, an excellent indicator of kidney function, in this study was marked by a non-significant decrease at the month 6 of ART. The month 6 creatinine levels were dependent upon the ART regimen used by the patient. This lack of significant increase in the creatinine levels between baseline and month 6 may indicate normal, functional and intact kidneys.

HIV infection contributes significantly in various degrees to immunopathogenesis in man [30]. Significant hematological and biochemical complications have been observed due to HIV. Abnormalities may occur in individuals as a result of the following actions; HIV infection, sequel of HIV-related opportunistic infections, malignancies and consequence of therapies used for HIV infection and associated conditions [31]. In this study, cases of thrombocytopenia, anemia, leucopenia and lymphocytopenia were observed. Other studies

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### Table 3: Regression models showing independent variables predicting changes in month 6 laboratory parameters.

<table>
<thead>
<tr>
<th>Model</th>
<th>Unstandardized Coefficients</th>
<th>95%Confidence Interval for B</th>
<th>Standardized Coefficients</th>
<th>t</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline CD4</td>
<td>0.44</td>
<td>0.07</td>
<td>0.31</td>
<td>0.57</td>
<td>0.027</td>
</tr>
<tr>
<td>Duration with HIV</td>
<td>10.10</td>
<td>4.27</td>
<td>1.71</td>
<td>18.48</td>
<td>0.10</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>0.711</td>
<td>0.08</td>
<td>0.56</td>
<td>0.86</td>
<td>0.37</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.088</td>
<td>0.04</td>
<td>0.00</td>
<td>0.17</td>
<td>0.09</td>
</tr>
<tr>
<td>ART regimen</td>
<td>Hb (g/dL)</td>
<td>0.00</td>
<td>0.04</td>
<td>0.04</td>
<td>0.13</td>
</tr>
<tr>
<td>Baseline Hb</td>
<td>0.122</td>
<td>0.04</td>
<td>0.04</td>
<td>0.20</td>
<td>0.13</td>
</tr>
<tr>
<td>WBC (10⁹/mm³)</td>
<td>0.851</td>
<td>0.02</td>
<td>0.82</td>
<td>0.89</td>
<td>0.91</td>
</tr>
<tr>
<td>Lymphocytes (10⁹/L)</td>
<td>0.885</td>
<td>0.02</td>
<td>0.85</td>
<td>0.92</td>
<td>0.93</td>
</tr>
<tr>
<td>Duration with HIV</td>
<td>-0.011</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>-0.04</td>
</tr>
<tr>
<td>Platelets (10⁹/L)</td>
<td>0.833</td>
<td>0.02</td>
<td>0.80</td>
<td>0.87</td>
<td>0.91</td>
</tr>
<tr>
<td>Baseline PLT</td>
<td>0.377</td>
<td>0.17</td>
<td>0.05</td>
<td>0.70</td>
<td>0.04</td>
</tr>
</tbody>
</table>

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have reported significant variation in the prevalence of hematological abnormalities in HIV participants, with anemia shown to range from 1.3% to 95% [32,33]. At the 6th month post ART initiation, thrombocytopenia, anemia, and leucopenia were reduced to 6.5%, 29.4% and 42.4% respectively while lymphocytosis increased marginally (2.5 to 5.3%). Our findings are similar to those of [14,34] who showed significant reduction in some hematological abnormalities due to ART. Although several research has shown that administration of ART especially Zidovudine (AZT) therapy causes anemia with a significant reduction in hemoglobin in HIV participants [35], our study was marked by increase in the mean hemoglobin levels at month 6 marked by reduction of anemia. Our results indicate that ART administration reverses the HIV associated anemia, a fact confirmed by Johannessen et al. [36]. The reduction in opportunistic infections including TB, as well as the reduction of inflammatory cytokines such as tumor necrosis factor (TNF) that are implicated in the suppression of erythropoiesis could be mechanisms that may account for the improvement of anemia after initiation of ART [37].

Our study showed that baseline hemoglobin level predicted the hemoglobin level at month 6. In other studies, stage of HIV, age and gender of the participants predicted the prevalence of anemia in HIV participants [38]. Despite a significant reduction in the prevalence of anemia at 6 months, close to 29.4% of the participants had anemia implying the need for routine screening of anemia and subsequent investigation of its causes.

As a practice, it is important to monitor the overall White Blood Cell (WBC) count because elevation of WBC may indicate infection, lack of response to treatment or an abnormality. In our study the mean WBC count was increased at month 6 during ART treatment. The progressive increase observed in absolute lymphocyte and total WBC may indicate a concerted suppressive activity of both immune system and the antiretroviral drug on the virus with the resultant decrease in leucopenia and lymphocytopenia. Leucopenia (a decrease in the number of white blood cells) and Lymphopenia (decrease in lymphocytes) are important hallmarks of HIV infection, and are also caused by certain medications such as ART [39,40] and certain infections [41]. Further investigation is needed to ascertain the mechanism responsible for leucopenia and lymphopenia among this cohort.

One of the major strength of this study was its design as a prospective study in a well-characterized cohort in which clinical outcomes were carefully measured and recorded. Some of the limitations of this study include; the short duration of follow up (6 months) and the relatively small sample size may have failed to detect smaller differences in some outcomes. The lack accurate ART adherence monitoring as well as the lack of other information such as nutritional status, use of herbal remedies during the study, or use of medications obtained outside the research clinic that may have influenced the observations of this study. The lack of plasma drug levels, ART drug potency, host pharmacodynamics and HIV drug resistance information which are important when evaluating the effectiveness of any ART call for a cautious interpretation of our results.

However, these limitations notwithstanding, the study has demonstrated that a robust immunological and virological response is achievable among participants receiving HIV treatment in the largest cohort of HIV positive population receiving HIV care and treatment in Nairobi Kenya. Further, it has shown that ART resulted in a remarkable reduction in the prevalence of adverse effects such (hepatic and renal impairment, thrombocytopenia, anemia, leucopenia and lymphocytosis) after 6 months. However, a substantial proportion of participants still had liver enzymes elevation and hematological abnormalities after 6 months of ART, raising the need for routine screening of biochemical and hematological outcomes, investigating theirs causes and instituting appropriate treatment and management strategies to mitigate the adverse effects of ART.

The most important determinant of the effectiveness of ART (improvement in CD4 count, achievement of viral suppression and reduction in adverse effects) were the baseline laboratory parameters at ART initiation. This data therefore suggests that earlier HIV diagnosis and initiation of ART in Kenya should be adopted in order to achieve optimal treatment outcomes.

Acknowledgement
We would like to thank the study participants enrolled at the FACES-KEMRI HIV care and treatment program for their invaluable support by consenting to take part in this study. We wish to acknowledge the Director KEMRI, Director CMR, and all the staff of the FACES Nairobi, Kenya.

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Author's Contribution
MON, FAO, WDB, CM, ANG, MO conceived the study, CM supervised sample collections while MON, FAO, WDB and MO supervised laboratory analysis. MON, FAO and MO analyzed the data and prepared the draft manuscript. FAO, WDB, ANG and MO provided guidance and mentorship during the implementation of the study. All authors reviewed and approved the final manuscript.

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