# Evaluation of the Impact of Delayed in Diagnosis and Initiation of Antiretroviral Therapy for HIV-1 Infected Infants on Their Viral Load (Viraemia) and CD4 T Lymphocyte Cell Status Outcome in the Gambia.

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# Abstract

**Background:** To enhance durable viral suppression and upgrade CD4 cell immune function of HIV-1 proviral DNA positive infants, Gambia government adopted WHO recommended sample collection from infants born to HIV infected mothers before or at 6-8 weeks soon after birth, then ensure 4 weeks' timeframe from collection dates, through molecular testing, to ART initiation if positive. Despite, studies to determine if these infants sample were collected, tested and initiated on ART within the adopted recommended timeframe, to ensure their achievements of this benefit are lacking.

**Aims:** We aimed to determine the effect of delayed in diagnosis and ART initiation on CD4 cell and viraemia outcome of HIV-1 proviral DNA positive infants in the Gambia.

**Method:** 2015-2019 retrospective data collection and analysis of key dates, initial viraemia and CD4 cell outcome, and then prospective cohort study on CD4 cell and viraemia outcome of those followed within at least 6 months and at most 3 years duration on ART adherence. STATA version 13 was used for the data analysis. Delayed in diagnosis and ART initiation was dichotomized using the adopted recommended timeframe, and Pair T-test used to determine the difference between mean initial and mean prospective, CD4 cell and viraemia outcome respectively.

**Results**: Between 2015-2019, 95 infants were found tested HIV-1 proviral DNA positive among which, 49/95 were found initiated on ART 42 weeks (IQR: 25, 83) median time from their delivery dates. Among these 49, 4 found adhered to the duration in the cohort of those not affect by the delays, difference between their mean initial and prospective CD4 cell outcome was found significantly (P = 0.02) higher than the 11 found adhered to the duration in the cohort of those affected by the delays (P = 0.37).The reverse was found in their viraemia outcome although, not statistically significant for both (P values = 0.33 and 0.18 respectively).

**Conclusion:** The overall 42 weeks  median time was found in conflict with the adopted recommended timeframe thus, found affected the CD4 cell and viraemia outcome of positive infants affected by the delays. Henceforth, the urgent attention is required for those affected to improve their prognosis.

# Introduction:

Sub-Saharan Africa, the region in which the Gambia is located, accounts for up to about 10% of the global population, but with over 79 % of the global HIV prevalence and about 92% and 90% prevalence in infected pregnant mothers and their infants respectively [1, 2]. In this region, over 80% of all under five HIV/AIDS related mortalities [3] among which, one-third died in the first year of life and half before 2 years, is attributed to either their lack of or poor access to early infant diagnosis (EID) of HIV-1 proviral DNA screening detection test, a bench-mark for rapid ART initiation of positive infants [1]. Therefore, ensuring this process (EID of HIV-1 proviral DNA screening detection for infants born to HIV infected mothers), for the benefits of immediate universal ART initiation of positive infants, together with ensuring their retention in care and treatment requires public health attention in the region. This is associated with

improve prognosis (durable viral suppression (viral load count) and upgrade the overall immune function (CD4 T lymphocyte cell)) and prevents or significantly reduces infancy or childhood HIV related morbidity and mortality risks [4, 5]. However, polymerization chain reaction (PCR) assay mainly utilize in HIV-1 proviral DNA screening detection test for below 18 months old infants/children born to HIV infected mothers, due to probable interference of persistent maternal antibodies with the use of HIV antibody (serological) or rapid tests, is challenge by cost, training and infrastructure requirements for availability or sustainable utilization in many laboratory settings in region [2, 6, 7]. Thus calls for collected samples transportation to central laboratories of majority of countries in the region for screening detection test, return of test results to collected health facilities/sites, together with notification of caregivers/mothers for the receipt of their infant's test results and immediate ART initiation if positive. For instance, in the Gambia, collected samples are transported to the central laboratory by a trekking team from the unit through the same processes described above. This significantly contributed to 51% of over 1.6 million African infants born to HIV infected mothers to have access the screening detection test in 2015 alone [8]. Consequently, bridged or delayed significant number of those who would be positive but were not tested, from benefiting WHO recommended immediate ART initiation of HIV-1 proviral DNA positively tested infants regardless, to immune status or clinical staging [4, 9–11]. Furthermore, the use of dry blood spot (DBS) is mainly utilized in HIV-1 proviral DNA screening detection test in the region, because it enables effective sample collections and preparations even at less equipped health facilities that only offers the collection services [2, 6, 12]. However, ensuring timely transportation of these samples to central/regional laboratories could be a challenge in the region. Nonetheless, current point-of-care (POC) molecular platform innovations to facilitate POC HIV-1 proviral DNA screening detection test, was found eased the burden of delayed in diagnosis and enables rapid ART initiation of positive infants, together with their increase retention in care and treatment [13]. Therefore, it could be anticipated that exploring their effective utilizations in the region, could ultimately ease the regional burden of delayed in diagnosis and ART initiation of positive infants, together with maintaining infected mothers-infants pair in care and treatment. Despite, HIV infected mothers who have not received or only received shortcut of ART (either not underwent or completed prevention of mother-to-child transmission of HIV infection services) are not only significantly contributing to MTCT of HIV-1 infection in the region, but also delayed sample collection from their high risk HIV expose infants [1, 4, 11, 12, 14]. Delayed in sample collection may delay screening detection test and ART initiation of their high risk expected positive infants. This ultimately could compromise their durable viral suppression (viral load count) and overall CD4 T lymphocyte cell immune function, together with increase in their mortality risks particularly, if they are not maintain in care and treatment as described above. Although, a decline in MTCT of HIV infections is observed in the region [15] however, the rate in the Gambia was 25% for HIV-1 and 4% for HIV-2 in 2000 [16]. This had committed the governments to prioritized HIV-1 proviral DNA screening detection test at the central laboratory of the country in 2015, because it was piloted in 2014 but screening test were done at Medical Research Council (MRC) unit the Gambia, at London School of Hygiene and Tropical Medicine. Despite national challenges in lack of HIV-2 proviral screening detection test capacity, thus a limitation for this study to include them, the prevalence of HIV-1 proviral DNA infections in 2016 was 6.4% (7/109), found significantly associated with the same characteristics of HIV infected mothers significantly contributing to MTCT in the region

described above [17]. Unfortunately, studies to determine if these infants (HIV-1 proviral DNA positives) DBS samples were collected, tested and initiated on ART within the adopted recommended timeframe as in many countries in the region, are lacking since the inception of the services in the Gambia. However, since ensuring these processes is crucial in maintaining them in care and treatment, significant associated with their improve prognosis (durable viral suppression (viral load count) and overall immune function (CD4 T lymphocyte cell) as described above, necessitated the study to inform decisions if these infants samples were collected, tested and initiated on ART within the adopted recommended timeframe and its effect on their CD4 cell and viraemia count outcomes if they weren't, for appropriated response mechanisms.

## Methodology:

### Study designand Population:

Retrospective and prospective cohort study, which involved retrospective data collection and analysis of key dates from deliveries to ART initiations and initial CD4 cell and viraemia count outcomes of all HIV-1 proviral DNA positive infants who had their samples collected between 2015 and 2019. Those found initiated on ART were enrolled in cohorts of study group (HIV-1 infected infants delayed in diagnosis and ART initiation, delayed in diagnosis but not in ART initiation, and delayed in ART initiation but not in diagnosis) and control group (HIV-1 infected infants neither delayed in diagnosis nor in ART initiation). less than or equals to (≤) 8 weeks between delivery dates and DBS sample collection dates (not delayed in diagnosis); less than or equals ≤ 4 weeks between sample collection dates and ART initiation dates (not delayed ART initiation); greater than (>) 8 weeks between delivery dates and sample collection dates (delayed in diagnosis) and greater than (>) 4 weeks between sample collection dates to ART initiation dates (delayed in ART initiation); was dichotomized using the adopted recommended timeframe described above. Those on ART adherence from initiated date in the study and control group, a simple direct descriptive analysis of difference between dates ART was initiated and dates whole sample should be collected from each between January and March 2020 was conducted. This was done to identified the eligible (at least 6 months to at most 3 years duration on ART adherence) prospective study participants. Participant's information sheet that entails the purpose, benefits, and procedures involved in the study together with inform consent form, was used to obtain consent from mothers/caregivers of the identified eligible participants and they were prospectively followed for their infant's CD4 cell and viraemia count whole blood sample collection. They had the authority to withdraw at any time or stage during the study without any implication.

### Study sites:

Fifteen (15) PMTCT-ART health facilities namely: SOS and Sibanor clinics; Serekunda, Banjulinding, Bajakunda and Janjangbureh major health centers; Brikama/Hand-on- Care, Soma, Essau and Basse district hospitals; Bansang, Bwiam, Serrekunda and Farafeni general hospital; and Edward Francis Small Teaching hospital; were accessed for the study .The sites were selected and accessed based on their

registration of at least one HIV-1 proviral DNA positively tested infants from the central and 4 study sites laboratories (Brikama/Hands-on-Care, Soma, Bansang and Basse) capacitated for the services.

## Sample size:

The sample size included all HIV-1 poviral DNA positive infants who had their samples collected from 2015 to 2019, whether death or alive.

## Inclusion criteria and exclusion criteria:

The retrospective study included all HIV-1 poviral DNA positive infants whether death or alive and had their samples collected between 2015 and 2019, while the prospective study included only consented mothers/caregivers of the identified eligible infants. However, HIV-1 infected infants who doesn't underwent proviral DNA screening detection test or did not have his/her samples collected from 2015 to 2019, whether death or alive were excluded in the entire study. Furthermore, unconsented mothers of eligible prospective study participants were also excluded.

## Sampling frame and method:

A standardized and ethically approved data collection form was used to extract the list of all HIV-1 proviral DNA positive DBS PMTCT/year numbers, collected health facilities name (center name) and date tested from the 5 testing laboratory records described in the study sites. This was used to track them at their respective collected site PMTCT ART and Infants ARV registers (PA&IAR) to extract: delivery dates; date samples were collected; date send to testing laboratories; date results received from testing laboratories and survival status. Although, information on every step and date were usually found not routinely collected and available in their records despite, services providers were informed and trained. However, where this instance was encountered, they were documented not available (NA)/missing data thus, a challenge for this study. For those found initiated on ART, treatment folders were reviewed to extract: date ART was initiated; initial CD4 cell and viraemia count results; together with ART status. They were stratified and ranked into study and control groups using the WHO national adopted recommended timeframe described above. The study group included three strata ( 1. those delayed in diagnosis and ART initiation, 2. those not delayed in diagnosis but delayed in ART initiation and 3. those not delayed in ART initiation but delayed in diagnosis), while the control group included one strata ( those neither delayed in diagnosis nor in ART initiation). Among these groups, those identified eligible and were consented as described in the study design and population above, 2 to 3 ml whole blood samples were collected from them by trained laboratory staff for immediate CD4 cell count and processing the whole blood sample to plasma for storage at -20 °C, as equally done in their routine procedures. Becton Dickinson and company (BD) FasCount machine, available at all ART sites were used for CD4 cell counts. This was to ensure the consistent used of machine previously used for their initial CD4 cell counts. The -20 °C stored samples were used for viraemia count by the researcher under the supervision of the laboratory Head. Abbott Real-Time m2000sp/rt PCR machine available only at central laboratory was used, also to ensure consistent used of machine previously used for their initial viraemia counts. Despite

sample were stored before viraemia counts was done, due to reasons described above. However, date whole blood samples were collected from each eligible study participant represented his/her CD4 cell and Viraemia count ART duration outcome from initiated date.

## Data analysis:

Collected data were entered in excel spread sheet, then imported in STATA (StataCorp LP, College Station, Texas, USA) version 13 software and cleaned for analysis. However, to examine the normality of continuous variables, histogram and quantile-quantile plots, together with Shipiro-Wilk test was used to ascertain the type of test applicable for each variable. This was because the variable time interval (turnaround tine) between key dates was not normally distributed due to, information's on every step and date was not routinely collected and available in their records as previously described. Therefore, median and interquartile (IQR) were reported for the time interval variables. Wilcoxon rank-sum test was used to compare the time intervals between deliveries and ART initiations, together with between sample collections and ART initiation, while difference in mean initial and mean prospective, CD4 cell and viraemia outcome respectively among the study and control groups were compared using Paired t-test. Turnaround time was dichotomized as: less than or equals to (≤) 8 weeks between delivery dates and DBS sample collected dates (not delayed in diagnosis); ≤ 4 weeks between DBS samples collected dates and ART initiation dates (not delayed in ART initiation); greater than (> 8) weeks between delivery dates and sample collected dates (delayed in diagnosis); and > 4 weeks between DBS samples collected dates and ART initiation dates (delayed in ART initiation). All the categorical variables were coded for the analysis and p-values less than 5% were considered statistically significant.

# Results:

Between 2015 and 2019, 95 infants from 15 PMTCT health facilities/clinics in the Gambia were found tested HIV-1 proviral DNA positive. The median turnaround time found from their delivery dates to date DBS samples were collected from them was 13 weeks (IQR: 7, 34), date DBS samples were collected to dates HIV-1 proviral screening detection test were done median turnaround time found was 6.5 weeks (IQR: 3, 37.5) and from the dates screening detection test were done to dates test results were received at ART clinics median turnaround time found was 1 week ( IQR: 1, 4). Furthermore, greater than half (49/95) of them were found initiated on ART among which, the media turnaround time found from the dates their test results were received at ART clinics to ART initiation dates was 3.5 weeks (IQR 0.5, 10.5). Overall, the median turnaround time found from dates their DBS samples were collected to ART initiation dates and from delivery dates to ART initiation dates was 12 weeks (IQR: 7, 49) and 42 weeks (IQR; 25, 83) respectively as characterized in Table 1.

Table 1

Characteristics of the overall 95 HIV-1 proviral DNA positive infants in the study population on key date turnaround time from their delivery to ART initiation dates.

| Variable (Turnaround time) | N = 95 |
|---|---|
| Between date of birth and DBS sample collection (weeks): median (IQR) | 13 (7, 34) |
| Between DBS sample collection and testing (weeks): median (IQR) | 6.5 (3, 37.5) |
| Between testing and results received at ART clinics (weeks): median (IQR) | 1 (1, 4) |
| Between results received at clinics and ART initiations (weeks):median (IQR) | 3.5 (0.5, 10.5) |
| Between DBS sample collection and initiation of ART (weeks):median (IQR) | 12 (7, 49) |
| Between date of birth and initiation of ART (weeks): median (IQR) | 42 (25, 83) |
| **NB**: IQR: interquartile range; ART: antiretroviral therapy; DBS: Dry Blood Spot; N: total number of HIV-1 proviral DNA positive infants in the study population on median turnaround time involved between their key dates (delivery and DBS sample collections; DBS sample collections and HIV-1 proviral DNA screening detection test; screening detection test and receipts of test results at ART clinics; receipts of test results at ART clinics and ART initiations, DBS sample collections and ART initiation; and delivery and ART initiation) | |

In accordance to the WHO national adopted recommended timeframe (before or at 6–8 weeks soon after birth DBS sample collection from infants born to HIV infected mothers and 4 weeks turnaround time from collected dates, through molecular testing and to ART initiation if positive) described in study design and population above, among the 49 infected infants who were found initiated on ART, 9 (18.4%) and 40 (81.6%) of them were found not delayed and delayed in ART initiation respectively (dichotomized in this study as they had been initiated on ART less than or equals to (≤) 4 weeks and greater than (>) 4 weeks respectively from their DBS sample collected dates). On the other hand, 19 (38.8%) and 30 (61.2%) were found not delayed and delayed in diagnosis (dichotomized in this study as they had their DBS samples collected less than or equals to (≤) 8 weeks and greater than (>) 8 weeks respectively from their delivery dates) as characterized in Table 2.

Table 2

Characteristics of the overall 49 HIV-1 proviral DNA positive infants initiated on ART in the study population found not delayed or delayed in ART initiation and not delayed or delayed in diagnosis respectively.

| Variable | N = 95 |
|---|---|
| ART initiation (weeks): median (IQR) | 12 (7, 49) |
| ≤ 4 weeks: n (%) | 9 (18.4) |
| > 4 weeks: n (%) | 40 (81.6) |
| Missing, n | *46* |
| Diagnosis (weeks): median (IQR) | 12 (7, 49) |
| ≤ 8 weeks: n (%) | 19 (38.8) |
| > 8 weeks: n (%) | 30 (61.2) |
| Missing, n | *46* |

**NB**: ART: antiretroviral therapy; Missing: (HIV-1 proviral DNA positive infants in the study population either with unknown ART status or not initiated on ART); IQR: Interquartile range; n: number and %: percentage of HIV-1 proviral DNA positive infants among the 49 initiated on ART in the study population found had less than or equals to (≤) 4 weeks (not delayed in ART initiations) and greater than (>) 4 weeks (delayed in ART initiations) from their DBS samples collected dates to ART initiation dates and those found had less than or equal to (≤) 8 weeks (not delayed in diagnosis) and greater than (>) 8 week ( delayed in diagnosis) from their delivery dates to DBS samples collected dates dichotomized in accordance to the WHO national adopted recommended timeframe (before or at 6−8 weeks soon after birth DBS sample collection from infants born to HIV infected mothers and 4 weeks turnaround time from collected dates, through molecular testing and to ART initiation if positive)

Table 3 below, described the representation of the 49 HIV-1 proviral positive infants found initiated on ART in study and control group in respect to the adopted recommended timeframe. These significantly constituted the three strata's of our study group (1. Those found delayed in diagnosis and ART initiation as 75.0% (n = 30); 2. Those found not delayed in diagnosis but delayed in ART initiation as 25.5% (n = 10); 3. Those found delayed in diagnosis but not delayed in ART initiation as 0.0% (n = 0)) in comparison to the lone strata of our control group (Those found neither delayed in diagnosis nor in ART initiation as 100.0% (n = 9)) as characterized in Table 4

Table 3

Representation of the overall 49 HIV-1 proviral positive infants found initiated on ART in study and control group in accordance to the adopted recommended timeframe.

| Variable | Group comparison | | P value |
| --- | --- | --- | --- |
| | Control group | Study group | |
| ART initiation: n (%) | | | < 0.001 |
| ≤ 4 weeks | 9 (100.0) | 0 (0.0) | |
| > 4 weeks | 0 (0.0) | 40 (100.0) | |
| Diagnosis: n (%) | | | < 0.001 |
| ≤ 8 weeks: | 9 (47.4) | 10 (52.6) | |
| > 8 weeks: | 0 (0.0) | 30 (100.0) | |

**NB**: ART: antiretroviral therapy; n: number and %: percentage of HIV-1 proviral DNA positive infants among the 49 initiated on ART in the study population found had ≤ 4 weeks from their DBS sample collected dates to ART initiated dates dichotomized not delayed in ART initiation (control group) in comparison to those found had > 4 weeks from their DBS sample collected dates to ART initiated dates dichotomized delayed in ART initiation(study group); together with those found had ≤ 8 weeks from their delivery dates to DBS sample collected dates dichotomized not delayed in diagnosis(control group) in comparison to those found had > 8 weeks from their delivery dates to DBS sample collected dates dichotomized delayed in diagnosis (study group).

Table 4

Characteristic of the overall 49 HIV-1 proviral positive infected infants found in the three (3) strata's of the study group and in the lone (1) strata of the control group.

| Variable | Diagnosis | | P value |
| --- | --- | --- | --- |
| | ≤ 8 weeks | > 8 weeks | |
| ART initiation: n (%) | | | < 0.001 |
| ≤ 4 weeks | 9 (100.0) | 0 (0.0) | |
| > 4 weeks | 10 (25.5) | 30 (75.0) | |

**NB**: ART: antiretroviral therapy; n: number and %: percentage of HIV-1 proviral DNA positive infants among the 49 initiated on ART in the study population found had ≤ 8 weeks and ≤ 4 weeks dichotomized not delayed in diagnosis and ART initiation respectively among the lone strata of the control group)); together with those found had > 8 weeks and > 4 weeks; ≤ 8 weeks and > 4 weeks; and > 8 weeks and ≤ 4 weeks dichotomized delayed in diagnosis and ART initiation (strata 1 of the study group); not delayed in diagnosis but delayed in ART initiation (strata 2 of the study group); and delayed in diagnosis but not delayed in ART initiation (strata 3 of the study group) respectively.

Furthermore, duration on adherence to ART status from initiated dates to the end of our data collection in March 2020 found among the same 49 infected infants initiated on ART in the control group strata in

comparison to study group strata's includes: 25 were found had greater than or equals to (≥) 6 months on ART adherence from initiated dates among which, 4 (16.0%) were found neither delayed in diagnosis nor in ART initiation (lone strata of the control group) and 21 (84.0) found either delayed in diagnosis and ART initiation or only in ART initiation (strata 1 and 2 of the study group); whereas, 12 (100.0%) were found defaulted from ART adherence of which, all of them were found among the same 2 strata's of the study group described above. Furthermore, 12 were found had less than (<) 6 months on ART adherence from initiated dates among which, 5 (41.7%) were found in the same lone strata of the control group and 7 (58.3%) found among the same 2 strata's of the study group as characterized in Table 5.

Table 5
Characteristics of the overall 49 HIV-1 proviral DNA positive infants found initiated on ART among the lone strata of the control group durations on ART adherence in comparison to those found among the remaining 2 strata's of the study group duration on ART adherence.

| Variable | Group comparison | | P value |
|---|---|---|---|
| ART adherence status: n (%) | Control group | Study group | |
| ≥6months on ART adherence | 4 (16.0) | 21 (84.0) | 0.03 |
| default from ART adherence | 0 (0.0) | 12(100.0) | |
| <6months on ART adherence | 5 (41.7) | 7 (58.3) | |

**NB**: ART: antiretroviral therapy; n: number and %: percentage of HIV-1 proviral DNA positive infants among the 49 initiated on ART in the study population durations on ART adherence from initiated date to the end of our data collection in March 2020 among the control group lone strata (those found neither delayed in diagnosis nor in ART initiation/ had their DBS samples collected ≤ 8 weeks from their delivery dates and initiated on ART ≤ 4 weeks from their DBS sample collected dates ) in comparison to study group remaining 2 strata's (those found either delayed in diagnosis and ART initiation or only in ART initiation/either had their DBS samples collected > 8 weeks from their delivery dates and initiated on ART > 4 weeks from their DBS samples collected dates or had their samples collected ≤ 8 weeks from their delivery dates but they were initiated on ART > 4 weeks from their DBS sample collected dates).

Among the 25 HIV-1 proviral DNA positives found ≥ 6 months on ART (identified eligible prospective study participants through a simple direct descriptive analysis of difference between dates ART was initiated and dates whole sample should be collected from each between January and March 2020 previously described in the study design and population above), 1 was found death after 6 month on ART adherence and 3 declined for the study, but they were all found among the remaining 2 strata's of the control group (those found either delayed in diagnosis and ART initiation or only in ART initiation/either had their DBS samples collected > 8 weeks from their delivery dates and initiated on ART > 4 weeks from their DBS samples collected dates or had their samples collected ≤ 8 weeks from their delivery dates but they were initiated on ART > 4 weeks from their DBS sample collected dates). The number and percentage of those found adhered to the duration (at least 6 months to at most 3 years duration on ART adherence from initiated) was all the 4 (26.7%) in the lone strata of the control group (those found neither delayed in diagnosis nor in ART initiation/ had their DBS samples collected ≤ 8 weeks from their delivery dates and

initiated on ART ≤ 4 weeks from their DBS sample collected dates ) and 11 (73.3%) out of the 21 in the remaining 2 strata's of the study group describe above. Whereas, 2 (100.0%) and 4 (100.0%) were found had less than (<) 6 months and greater than (>) 3 years on ART adherence, because they had their whole blood sample collected before and after their due dates respectively. Therefore, they were not included in the group comparison on effects of delays on CD4 cell and viraemia count outcome despite, they were found among the remaining 2 strata's of the control group described above. Overall, 21 HIV-1 proviral DNA positive infants mothers out of the 25 identified eligible prospective study participants were consented and had their whole blood sample collected and tested for CD4 cell and viraemia counts.

Table 6

Characteristics of the consented 21 HIV-1 proviral DNA positive infants found in the study and control group, on number and duration on ART adherence from initiated dates to dates their whole blood samples were collected between January and March 2020.

| Variable | Group comparison | | P value |
|---|---|---|---|
| | Control group | Study group | |
| ART adherence durations: mean ± SD | 12.8 ± 5.4 | 20.7 ± 13.6 | 0.70 |
| < 6 months: n (%) | 0 (0.0) | 2 (100.00 | |
| ≥ 6 months to ≤ 36 months n (%) | 4 (26.7) | 11 (73.3) | |
| > 36 months: n (%) | 0 (0.0) | 4 (100.0) | |

**NB**: ART: antiretroviral therapy; SD: standard deviation n: number and %: percentage of HIV-1 proviral DNA positive infants among the 21 consented identified eligible prospective study participants in the study population found adhered to the durations (at least 6 months to at most 3 years) on ART adherence from initiated dates to the end of our data collection in March 2020 among the control group lone strata (those found neither delayed in diagnosis nor in ART initiation/ had their DBS samples collected ≤ 8 weeks from their delivery dates and initiated on ART ≤ 4 weeks from their DBS sample collected dates ) in comparison to study group remaining 2 strata's (those found either delayed in diagnosis and ART initiation or only in ART initiation/either had their DBS samples collected > 8 weeks from their delivery dates and initiated on ART > 4 weeks from their DBS samples collected dates or had their samples collected ≤ 8 weeks from their delivery dates but they were initiated on ART > 4 weeks from their DBS sample collected dates).

Among those found adhered to the duration in the control group (4) and study group (11) as described above, the study found mean initial and prospective CD4 cell counts together with their difference, constantly much higher respectively in the control group than the study group. The reverse is found in their viraemia counts respectively too although, with difference in their mean CD4 cell count outcome described above only statistically significant in the control group (p-value = 0.02), while the difference in mean viraemia count outcomes was found not statistically significant in both groups as described in Table 7.

Table 7

Mean initial and prospective, CD4 cell and viraemia count, together with their difference respectively comparison between the 4 found adhered to duration in the lone strata of the control and 11 found in the 2 strata's of the control group.

| Variable | Group comparison | | | |
|---|---|---|---|---|
| CD4: mean (SE) | Control group | P value | Study group | P value |
| Initial CD4 count | 1435.5 (106.6) | 0.02 | 1032.2 (135.4) | 0.37 |
| Prospective CD4 count | 980.3 (148.2) | | 828.2 (156.0) | |
| Difference in CD4 count | 455.3 (100.9) | | 204.0 (207.6) | |
| Viraemia: mean (SE) | | 0.33 | | 0.18 |
| Initial viraemia count | 21098.5 (20681.5) | | 353342.3 (175342) | |
| Pros. viraemia count | 565567.1 (325683.8) | | 586571.2 (252636) | |
| Difference in viraemia | -544468.6 (305002.3) | | -233228.8 (114402.6) | |

NB: SE: standard error; CD4: Cluster of Differentiation thymus lymphocyte cells type 4 and Viraemia (HIV viral load count) mean difference between the initial and prospective count outcome among the 4 HIV-1 proviral DNA positive infants in control group lone strata (those found neither delayed in diagnosis nor in ART initiation/ had their DBS samples collected ≤ 8 weeks from their delivery dates and initiated on ART ≤ 4 weeks from their DBS sample collected dates ) in comparison to their difference among the 11 in the study group remaining 2 strata's (those found either delayed in diagnosis and ART initiation or only in ART initiation/either had their DBS samples collected > 8 weeks from their delivery dates and initiated on ART > 4 weeks from their DBS samples collected dates or had their samples collected ≤ 8 weeks from their delivery dates but they were initiated on ART > 4 weeks from their DBS sample collected dates).

# Discussion:

In our dichotomized WHO's national adopted recommended timeframe (before or at 6−8 weeks soon after birth DBS sample collection from infants born to HIV infected mothers and 4 weeks turnaround time from collected dates, through molecular testing and to ART initiation if positive) multivariable analysis of key dates from sample collection to ART initiation of HIV-1 proviral DNA positive infants in the Gambia, the median turnaround time found between their deliveries and DBS sample collections (13 weeks; approx.3.3 months), together with the overall median turnaround time found between sample collection dates and ART initiation dates (12 weeks- approximately 3 months) as described in Table 1, demonstrated mothers/caregivers of this infants took approximately twice (13/8 median time) the adopted recommended timeframe before their infant's samples were collected and health service logistics to facilitated the collected samples transportation to testing laboratories; performance of the screening detection test; return of test results to ART clinics; and to the caregivers/mothers for immediate ART initiation of their infected ones; was found trice (12/4 ) the adopted recommended timeframe. Therefore, the overall median turnaround time found between deliveries and ART initiations (42 weeks approximately 10.5 months) as described in Table 1, demonstrated both maternal and health service logistic delivery gaps were found contributed to delay in diagnosis and ART initiation of HIV-1 proviral

DNA positive infants in the Gambia. This is observed similar to findings of a study report in Ethiopia (both maternal and health system factors contributed to delaying the receipt of HIV test results because mothers/caregivers fail to bring their exposed infant for testing before or at 6–8 weeks soon after birth and there was delays in sample collection, sample transport to the regional laboratory center, performance of the HIV test and return of the test result to the clinic and patient) [1]. Necessarily, HIV infected mothers who have not received or only received shortcut of ART (either not underwent or completed PMTCT), are significantly found associated with delayed sample collection from their high risk HIV exposed infants [1, 12]. Delayed in their sample collections, could ultimately delayed their ART initiation. Therefore, taken in account of the 13 weeks median time discussed above, together with the 6.4% (7/109) prevalence in HIV-1 proviral DNA positives infants in the Gambia found attributed to mothers with such characteristic(s) [17].. Then it could be anticipated that significant number of these 95 HIV-1 proviral DNA positive infants found by our study, mothers had conceived either not received or only received shortcut of ART. Although, this may require further studies however, it could be found significantly demonstrated among the 49/95 HIV-1 proviral DNA positive infants found initiated on ART, with only 9 (38.8%) found not delayed in diagnosis and ART initiation (had their samples collected ≤ 8 weeks from delivery dates and were initiation on ART ≤ 4 weeks from collection dates) in comparison to 30 found delayed in diagnosis and ART initiation (had their samples collected > 8 weeks from their delivery dates and were initiated on ART > 4 week from collection dates), and 10 found only delay in ART initiation (had their samples collected ≤ 8 weeks from delivery dates but were initiated on ART > 4 weeks from collection dates) dichotomized using the adopted recommended timeframe described in Table 4. However, delayed in diagnosis and ART initiation of HIV-1 proviral DNA positive infants, are significantly attributed to increase their chances of progression to AIDS and mortality before the age of 2 years [1, 6, 15]. progression to AIDS or antiretroviral drugs resistant in HIV infected persons, could simply be conceived or clinically be confirm by lower CD4 cell count and primarily, higher viraemia count (HIV viral load count) outcome over a period on ART adherence, when compare to initial/pre-ART outcome. Therefore, HIV-1 proviral positive infant not affected by delayed in diagnosis and ART initiation could ultimately have higher CD4 cell and lower viraemia count outcome when compare to those affected despite, with equal duration on ART adherence. These was found symbolized by our findings on difference between mean initial and mean prospective CD4 cell outcome significantly higher among the 4 found not affected by the delays (control group; P value = 0.02) ) than the 11 found affected ( study group; P value = 0.37), while the difference in viraemia counts was found lower among the former than the latter. Although, the outcomes of their viraemia was not statically significant in both groups as described in Table 7, together with our findings limitations of data on the receipts of antiretroviral drugs (ARV, s) or prophylaxis by these positive infants after their deliveries, their feeding methods or nutritional status and genetic make-ups. However, if diagnosis and ART initiation were not delayed, there couldn't be statistical insignificant difference between mean initial and prospective, CD4 cell and viraemia outcome respectively among only the 11 HIV-1 proviral DNA positive infants in the study group remaining 2 strata's (those found either delayed in diagnosis and ART initiation or only in ART initiation/either had their DBS samples collected > 8 weeks from their delivery dates and initiated on ART > 4 weeks from their DBS samples collected dates or had their samples collected ≤ 8 weeks from their delivery dates but they were

initiated on ART > 4 weeks from their DBS sample collected dates) when compared to the 4 in the control group lone strata (those found neither delayed in diagnosis nor in ART initiation/ had their DBS samples collected ≤ 8 weeks from their delivery dates and initiated on ART ≤ 4 weeks from their DBS sample collected dates) despite, the sum of their duration on ART adherence was higher than 4 in the control group lone strata.

## Conclusion:

The overall median turnaround time (42 weeks) found between their delivery dates to ART initiation dates, attributed to maternal and health service delivery gaps, was found in serious conflict with the overall adopted recommended 12 weeks' timeframe. Thus, delayed diagnosis and ART initiation of significant number of HIV-1 proviral DNA positive infants in the Gambia and affected the enhancement of their durable viral suppression (viraemia/viral load count) and overall CD4 T lymphocyte cell immune functions.

Henceforth, the urgent attention is required for those affected by the delays to ensure their better viral suppression (viraemia/viral load count) and overall CD4 T lymphocyte cell immune functions. Furthermore, logistics to ensure adherence to the adopted recommended timeframe must be prioritize in the Gambia.

## Abbreviations:

HIV: Human Immunodeficiency Virus

AIDS: Acquired Immunodeficiency Virus

WHO: World Health Organization

MRC: Medical Research Council

PMTCT: Prevention of Mother-To-Child Transmission

MTCT: Mother-To-Child Transmission

EID: Early Infant Diagnose

ART: Antiretroviral Therapy

DNA: Deoxyribonucleic Acid

PCR: Polymerization Chain Reaction

CD4 T lymphocytes: Cluster of Differentiation thymus lymphocyte cells type 4

DBS: Dry Blood Spot

POC: Point-of-Care

ARV; Antiretroviral drug

PA&IAR: PMTCT ART and Infants ARV Register

# Declarations:

## Ethical consideration:

The study was reviewed and approved by Research and Publication Committee (RePubliC), School of Medicine and Allied Sciences, University of the Gambia (Ref no. R019007v2); then finally reviewed and approved by the Gambia Government/MRC Joint Ethics Committee with the same reference number. Furthermore, approval was also obtained from the Gambia Ministry of Health to access the study sites. Participant's information sheet that entails the purpose, benefits, and procedures involved in the study together with inform consent form, was used to obtained consent from all mothers/caregiver of HIV-1 infected infants who were enrolled in the prospective study. They had the authority to withdraw at any time or stage during the study without any implication.

## Consent to publication:

Not applicable

## Availability of data and materials:

Existing study codes (PMTCT numbers) for every study participants from their records were used in the entire study data collection and analysis processes. Collected and analyzed data were further stored in a media storage devise with strong password manage by researcher to limited access to the gathered data. However, this data could be access from the National Public Health Laboratories data achieves and the coded supporting data set could be made available the researcher upon request.

## Competing interest:

No financial or non-financial conflict of interest is undertaken by the researcher, thus no conflict of interest is required by the researcher in respect to universal access for future study, but must be citied accordingly as indicate in the journal.

## Funding:

Part of the funding for the study was obtained from the office of National AIDS Secretariat (NAS) under the office of the president, through the office of National AIDS Control Program (NACP), while the other part was facilitated through the researcher's stipend payment from the Gambia government, through the personnel management office (PMO).

## Authors' contributions:

MC, AHA, NN and BS, conceived the research idea, designed the study; MC, BS contributed to the analysis and interpretation of data and led the writing of the manuscript. MLJ, AM, contributed in the entire data collection verification and confirmation in addition to guiding the viraemia count processes, MN supported in guiding and supporting data analysis and AHA, NN, BS, EU, POB, IG and SJ made intellectual contribution to drafting or revising of the manuscript. All authors read and approve the final manuscript.

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# Supplementary Files

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