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Comparison of dried blood spot (DBS) and plasma HIV-1 viral load measurements using Roche COBAS AmpliPrep/COBAS TaqMan assay, Northwest Ethiopia

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Abstract

Introduction Quantitative determination of HIV-1 viral load measurements using plasma samples has been widely applicable for prompt monitoring at baseline and following initiation of highly active antiretroviral therapy (HAART). However, improper mixing of whole blood with anticoagulants during plasma sample processing, as well as limited access to specialized health facilities might hinder HIV diagnosis services. Considering its higher stability and increased accessibility in areas with poor laboratory settings, the dried blood spot (DBS) sample might be a suitable alternative approach for periodic monitoring of HIV-1 viral load measurements. Thus, in this study, we aimed to compare the quantitative determination of HIV-1 RNA levels using plasma and DBS samples among people living with HIV in Northwest Ethiopia.

Methods An institutional-based analytical cross-sectional study was conducted from March to July 2020 using 48 paired plasma and DBS samples among people living with HIV at the HIV Treatment Center, Northwest Ethiopia. A total of four milliliters of venous blood was collected to harvest plasma and for DBS sample preparation. The HIV-1 RNA extraction, amplification, and quantification were performed using the Roche COBAS AmpliPrep/COBAS TaqMan version 2.0 assay. Data were managed and analyzed using SPSS version 26 software. Mean HIV-1 viral load measurements as well as the associations between plasma and DBS sample measurements were computed using a paired sample *t*-test and Pearson's correlation statistical tests, respectively. In addition, the level of agreement and the presence of proportional bias between sample measurements were performed using the Bland–Altman plot and linear regression models, respectively. A p-value of ≤ 0.05 with a 95% confidence interval was considered statistically significant.

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Results Among 48 people living with HIV, more than half (64.6%) of them were females. The minimum and maximum age of the study participants was 12 and 58 years, respectively. The mean difference with standard deviation (SD) of sample measurements (DBS minus plasma) HIV-1 viral load was 0.66 ± 0.70 log copies/mL. In the current study, a strong association with a significant linear correlation ($r = 0.796$) ($p < 0.001$) was obtained from Pearson's correlation analysis among HIV-1 viral load measurements between DBS and plasma samples. Moreover, the Bland–Altman plot also depicted a high level of agreement between the sample measurements.

Conclusions The findings of the current study suggested that DBS samples could be considered as an alternative approach for periodic monitoring of HIV-1 viral loads to scale-up the HIV diagnosis and treatment coverage, particularly in areas with limited laboratory settings due to minimal invasive blood collection, higher stability at room temperature or ease of transportation, and decentralized sample collection approaches.

Keywords Human immunodeficiency virus, HIV viral load, Plasma, Dried blood spot, Northwest Ethiopia

Introduction

Human immunodeficiency virus (HIV) remains one of the most challenging infectious diseases worldwide and poses a significant public health impact, especially in people living in low and middle-income countries [1, 2]. Globally, an estimated 39.9 and 1.3 million individuals were living with HIV and acquired new infections, respectively as per the 2023 World Health Organization's (WHO) report [3]. Sub-Saharan countries remain the most severely affected territories, which claimed an estimated 29.1 million HIV cases worldwide. Particularly, in Ethiopia the number of people living with HIV was around 610 thousand at the end of the year 2023 [3, 4].

Prompt monitoring of HIV viral load is the most commonly used diagnostic platform to determine the efficacy of highly active antiretroviral therapy (HAART) and to predict the emergence of drug resistance among people living with HIV [5–7]. In addition, HIV viral load determination after six months of HAART initiation is particularly an indicator of successful viral suppression or a state of treatment failure (progression of HIV infection) [8, 9]. To reduce the global burden of HIV infection, WHO adopted a goal, which declares that by the end of the year 2030, 95% of people living with HIV will have access to a comprehensive diagnosis service. And 95% of those will have received a life-saving antiretroviral medication, as well as 95% of those who are on treatment will have attained a suppressed HIV viral load [10, 11].

HIV viral load measurements using plasma samples are the most commonly used diagnostic method and are considered the gold standard for periodic monitoring of quantitative HIV ribonucleic acid (RNA) among people living with HIV receiving antiretroviral therapy (ART) [12, 13]. However, the comprehensive diagnosis of HIV drug resistance genotyping and treatment coverage services might be affected by interference due to improper mixing of whole blood and anticoagulants during plasma sample processing, the need for cold chain for proper storage and transportation, as well as limited access to specialized facilities among countries with resource-limited settings [14, 15]. Thus, dried blood spot (DBS) samples might be a suitable alternative sample for the determination of HIV viral load determination before and following the initiation of ART regimens; due to their higher stability, no need for special laboratory processing, and ease of transportation at ambient temperature. In addition, studies also reported that the DPS samples might be an alternate sample source for viral load testing with a treatment failure threshold (1,000 copies/ml or 3.0 log10 copies/mL) as plasma specimens [7, 16]. However, studies that show concordance of HIV viral load measurements using DBS over plasma samples are limited, particularly in Ethiopia. Therefore, the current study aimed to compare the quantitative determination of HIV-1 viral load results obtained from plasma and DBS samples analyzed using the Roche COBAS AmpliPrep/COBAS TaqMan version 2.0 assay in northwest Ethiopia.

Materials and methods

Study design, period, and setting

An institutional-based analytical cross-sectional study was conducted from March to July 2020 among people living with HIV receiving ART at the University of Gondar Comprehensive Specialized Hospital (UoG-CSH)'s HIV Treatment Center, Gondar, Northwest Ethiopia. The institution (UoG-CSH) is located in the Central Gondar Zone of the Amhara Regional state, around 750 km from the capital city of Ethiopia (Addis Ababa). There are around 8 health centers, 2 private hospitals, near to 42 private clinics, and a single comprehensive specialized and referral hospital in the town. The UoG-CSH is a teaching hospital that provides comprehensive specialty and referral services to more than seven million individuals who live in Gondar town and its catchment area. Nowadays, the UoG-CSH provides services for around five thousand six hundred people living with HIV at the UoG-CSH HIV Treatment Center.

Source and study populations

All people living with HIV who were on ART at the UoG-CSH HIV Treatment Center were considered our source population. Whereas, people living with HIV of any age and who were receiving ART for more than six months at the UoG-CSH HIV Treatment Center were included as part of the study population. While, people living with HIV who had a severe illness and difficult to obtain a blood sample, and those individuals who attended ART service for less than six months were not considered for the current study.

Sample size determination and sampling procedure

The G-power software, version 3.1.9.7 [17] was applied to calculate the sample size for the current study. A paired sample *t*-test approach was aimed to compute the mean difference between two matched samples considering a medium effect size ($d = 0.3$), a significant level of 5% ($\alpha = 0.05$), and a desired power of 80% ($1\text{-}\beta = 0.80$) to obtain the ultimate sample size of 48 study participants. Then, a simple random sampling technique was applied to recruit people living with HIV who were receiving ART at the UoG-CSH HIV Treatment Center during the study period (March to July 2020).

Operational definition

Adherence

Measuring the level to which people living with HIV receiving ART comply with their treatment regimen.

Good adherence

95% adherence to HIV drugs, or less than 2 missed doses of 30 ART drug doses, or less than 3 missed doses of 60 ART drug doses [18, 19].

Poor adherence

less than 85% HIV drug adherence, or at least 6 missed doses of 30 ART drug doses, or more than 9 missed doses of 60 ART drug doses [18, 19].

Data collection

All the relevant data including sociodemographic and clinical characteristics were collected from patient's charts and their medical records. A well-trained clinical nurse professional who worked at the UoG-CSH HIV Treatment Center collected the data using a structured data collection form (S1 Table). Information on HIV treatment adherence including frequency of missed doses was obtained using a mixed-methods approach, such as a standardized questionnaire, reviewing their medical record and/or patient's charts.

Blood sample collection and processing

Four milliliters (4 mL) of venous blood was collected from people living with HIV by an experienced laboratory technologist using a vacutainer tube containing EDTA anti-coagulant. Of this, around 50 µL blood was dispensed to the Whatman 903 filter paper cards (Whatman, Sanford, CA, USA) on each of the five spots [20]. The blood spots were left to dry at room temperature for about 4–6 h and then, placed in zip-lock plastic bags with silica gel desiccant pouches. Plasma was harvested from whole blood samples through centrifugation for 10 min at 2500 revolutions per minute (RPM). Then, an aliquot of plasma was prepared using cryovials. The aliquots of plasma and the prepared DBS samples were stored at − 20 °C until they were tested for the quantitative HIV-1 RNA copies.

HIV-1 RNA extraction from DBS and plasma samples

The isolation of HIV-1 RNA from DBS samples was performed using around two half-spots (6 millimeters in diameter) trimmed from each filter paper card. Then, transferred to a 50 mL conical tube containing lysis solution by clean forceps. The COBAS AmpliPrep instrument (Roche Molecular Systems, Inc., Branchburg, NJ, USA) was used for the automated specimen processing from aliquots containing 1100 µL of plasma, DBS, negative control, HIV-1 low positive control, and HIV-1 high positive control samples. The HIV-1 RNA was extracted based on the silica-based capture principle from aliquots of plasma and DBS samples. A known concentration of HIV-1 Quantitation Standard (QS) RNA molecules (Ambion, Inc. and Cenetron Diagnostics, LLC., Branchburg, NJ, USA) was processed along with plasma and DBS samples. The target HIV-1 RNA and HIV-1 QS were isolated from impurities through their non-specific binding capacity to the magnetic glass particle surface. Then, the RNA molecules were eluted from the magnetic glass surface using aqueous solutions and prepared for the quantification step [21, 22].

Quantification of HIV-1 RNA from DBS and plasma samples

For quantification, the target HIV-1 RNA and HIV-1 QS RNA samples were added to an amplification mixture containing buffer solution, manganese (Mn^{2+}), and deoxynucleotide triphosphates (dNTPs) following extraction. Then, transferred to the COBAS TaqMan version 2.0 Analyzer (Roche Molecular Systems, Inc., Branchburg, NJ, USA). The HIV-1 RNA and HIV-1 QS RNA molecules were reverse-transcribed and amplified using a thermostable DNA polymerase. The complementary DNA denaturation and primer annealing steps were performed at 94 °C and 54 °C, respectively. Then, DNA extension was employed at 72 °C to produce a double-stranded complementary DNA molecule. The HIV-1

RNA and HIV-1 QS RNA-specific short sequences of nucleotide probes tagged with a fluorescent dye were used for detection and fluorescent signals were interpreted using the AMPLILINK version 3.4 software. The HIV-1 viral load measurements from plasma and DBS samples were interpreted as a target not detected and within the quantitative range of 20–10^7 copies/mL [21, 22].

Data management and quality control

A well-trained clinical nurse professional collected sociodemographic and clinical data, while an experienced laboratory technologist collected blood samples. Sample collection, processing, and laboratory testing were performed per the optimized standard operating protocols. In addition, all the laboratory assays were done according to the manufacturer's instructions and following the standard operational procedures. The negative controls, HIV-1 low positive, and HIV-1 high positive controls were processed along with plasma and DBS samples to assess the quality of our sample processing. To ensure the reliability and accuracy of the obtained results, all the laboratory assay processing steps were done under a controlled laboratory condition using a class II biosafety cabinet and through strict adherence to the national and international laboratory safety guidelines [23, 24].

Data analysis

The Statistical Package for Social Science (SPSS) version 26 software was used for data management and analysis. Means and cross-tabulation (chi-square) tests were applied to characterize the study participants and compare the quantitative HIV-1 viral load measurements among the paired samples. A paired sample *t*-test and Pearson's correlation analysis were computed to compare the mean difference and association between paired sample measurements, respectively. In addition, the Bland–Altman plot analysis and linear regression were applied to evaluate the level of agreement and proportional bias between sample measurements, respectively. Then, a *p*-value of ≤ 0.05 with a 95% confidence level was interpreted as statistically significant.

Table 1 Sociodemographic and clinical characteristics of study participants

Variables	Category	Frequency (*n*)	Percentage (%)
Sex	Male	17	35.4
	Female	31	64.6
Age (years)	≤ 17	9	18.8
	18–35	12	25
	≥ 36	27	56.3
HIV clinical stage	I	43	89.6
	II	5	10.4
Treatment regimen	First line[a]	32	66.7
	Second line[b]	16	33.3
Treatment adherence	Good	44	91.7
	Poor	4	8.3

[a]: adults who were treated with ABC + 3TC + EFV or TDF + 3TC + DTG and children treated with ABC + 3TC + LPV/r or ABC + 3TC + DTG. [b]: adults who were treated with TDF + 3TC + LPV/r or TDF + 3TC + DTG and children treated with ABC + 3TC + LPV/r or TDF + 3TC + EFV. ABC: abacavir, 3TC: lamivudin, EFV: efavirenz, TDF: tenofovir disoproxil fumarate, LPV/r: lopinavir/ritonavir, DRV/r: darunavir/ritonavir, HIV: human immunodeficiency virus

Results

Socio-demographic and clinical characteristics of study participants

In the current study, we included a total of 48 people living with HIV who were on ART to obtain paired DBS and plasma samples. The majority, 64.6% (31) of the individuals living with HIV were females. The mean age of people living with HIV was 34.1 years with a standard deviation (SD) of ± 11.6 years. The minimum and maximum age of people living with HIV were 12 and 58 years, respectively. More than half (56.3%) of the study participants were above 36 years of age, followed by 18–35 years of age (25%). Regarding their clinical stage, forty-three (89.6%) of them were under the stage I HIV clinical classification. Moreover, 66.7% (32) and 91.7% (44) of the study participants were receiving a first-line treatment regimen and had good treatment adherence, respectively (Table 1).

Quantitative HIV-1 RNA levels in DBS and plasma samples

A total of 48 (100%) paired plasma and DBS samples were obtained for the quantitative analysis of HIV-1 viral load results. Among the DBS HIV-1 viral load measurements, 34 (70.8%) and 4 (8.3%) of the samples were detected within the quantitative range of 20–1000 copies/mL (1.30–3.0 log copies/mL) and > 1000 copies/mL (> 3.0 log copies/mL), respectively. In addition, 21 (43.8%) and 3 (6.3%) of the plasma HIV-1 viral load results were detected between 20 and 1000 copies/mL (1.30–3.0 log copies/mL) and > 1000 copies/mL (> 3.0 log copies/mL), respectively. Whereas, 10 (20.8%) and 24 (50%) of the included paired DBS and plasma samples demonstrated undetected HIV-1 viral load results, which were below the low detection limit of the Roche COBAS AmpliPrep/COBAS TaqMan assay (20 copies/mL; <1.30 log copies/mL) (Table 2).

Comparison of quantitative HIV-1 viral load in plasma and DBS samples

A paired sample t-test was applied to compare the mean difference of HIV-1 viral load measurements from DBS and plasma samples. The mean difference with 95% confidence interval (CI) of quantitative HIV-1 RNA level between the DBS and plasma measurements was 0.66 (0.46–0.87) log copies/mL. The association between the two sample measurements was computed using Pearson's correlation analysis. A strong association with a

Table 2 HIV-1 viral load determination in paired DBS and plasma using the Roche COBAS AmpliPrep/COBAS TaqMan assay

	Plasma HIV-1 viral load (*n* = 48)				
DBS HIV-1 viral load (*n* = 48)		Not detected[a]	20–1000 copies/mL[b]	> 1000 copies/mL[c]	Total
	Not detected[a]	10	0	0	10
	20–1000 copies/mL[b]	14	20	0	34
	> 1000 copies/mL[c]	0	1	3	4
	Total	24	21	3	48

[a]: below the low detection limit, < 20 copies/mL (< 1.30 $\log_{10}$ copies/mL); [b]: 20–1000 copies/mL (1.30–3.0 $\log_{10}$ copies/mL); [c]: >1000 copies/mL (> 3.0 $\log_{10}$ copies/mL). The quantitative HIV-1 viral load levels between paired plasma and DBS samples were categorized to target not detected or below the low detection limit, 20–1000 copies/mL, and > 1000 copies/mL. Cross-tabulation was performed to show the quantitative agreements or relationships between plasma and DBS sample measurements. DBS: dried blood spot, HIV: human immunodeficiency virus

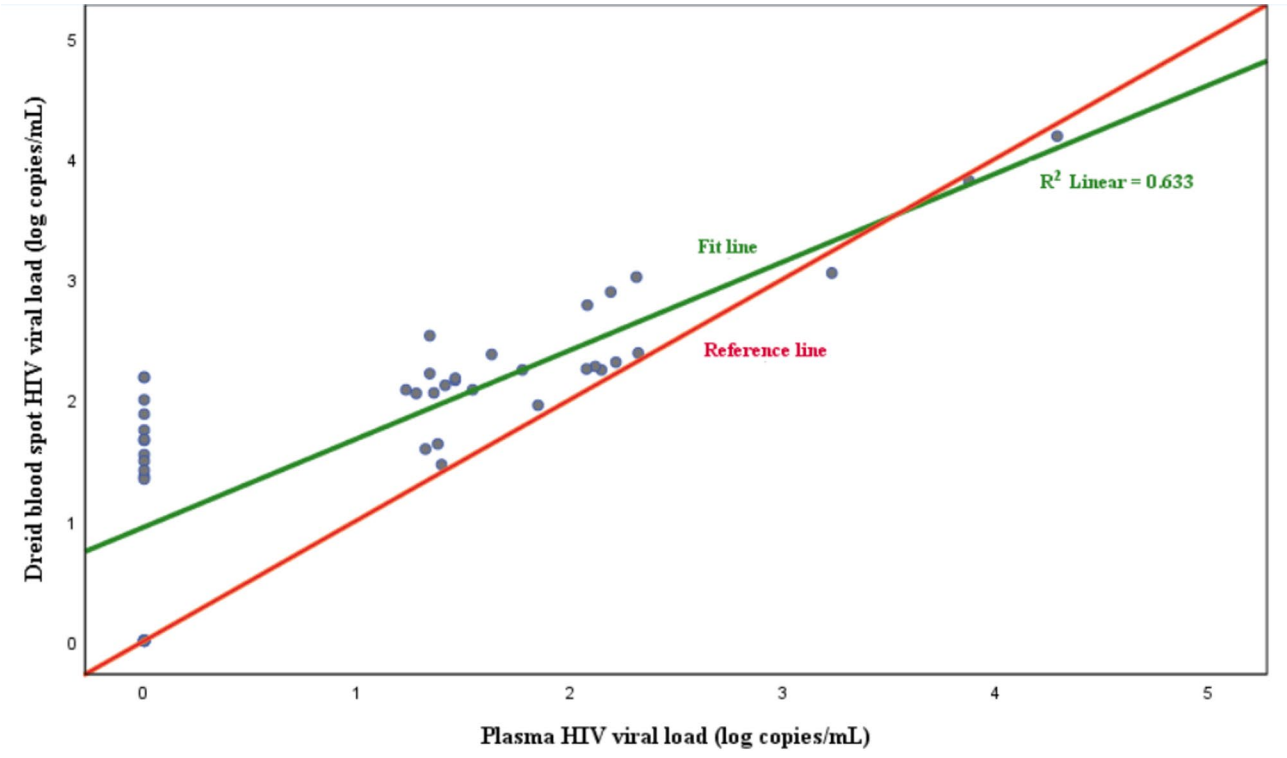

Fig. 1 Pearson's correlation analysis of HIV-1 RNA level in paired DBS and plasma samples. HIV-1 viral load was determined using 48-paired plasma and DBS samples. Pearson's correlation analysis was applied to demonstrate the association between HIV-1 viral load results obtained from DBS and plasma. The DBS and plasma HIV-1 viral load results (log copies/mL) were plotted in the vertical (y-axis) and horizontal (x-axis), respectively. Each dot on the scatterplot denotes a single data point. A fit line was drawn straight through the scatterplot to depict the linear relationship between the two variables and the data points closely clustered around the fit line were visually considered to have a strong correlation

significant linear correlation was observed between paired DBS and plasma HIV-1 viral load measurements ($r = 0.796$) ($p < 0.001$) (Fig. 1).

Evaluation of the agreement between plasma and DBS HIV-1 RNA measurements

The level of agreement between the two paired sample HIV-1 viral load measurements was assessed using the Bland–Altman analysis test. The difference plot for the HIV-1 viral load results shows that the mean difference was 0.66 (95% CI; −0.72–2.04) log copies/mL between the DBS and plasma measurements. As demonstrated in the Bland–Altman plot, there was a high level of agreement between the DBS and plasma HIV-1 viral load measurements (Fig. 2). In addition, no significant proportional bias was observed in the current sample measurements after analysis using linear regression ($p = 0.360$).

Discussion

HIV infection continues to have devastating consequences on individuals across the globe [25]. The most efficient approach for managing individuals with HIV is through the quantification of HIV RNA [26]. Sub-Saharan African countries have continuously overcome challenges to initiate and scale up HIV viral load testing programs to periodically monitor people living with HIV receiving ART. However, the level of HIV viral load monitoring coverage is still inadequate, which ranges from

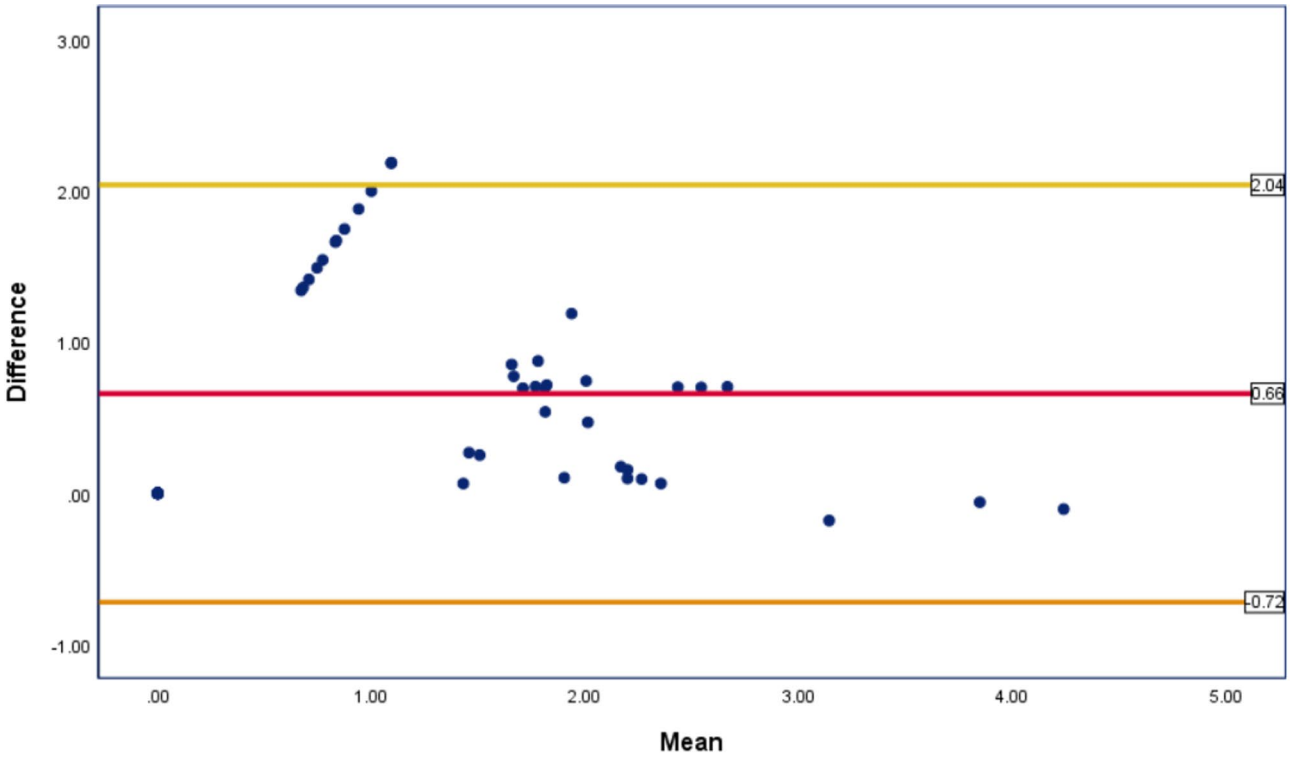

Fig. 2 The Bland–Altman plot analysis of paired DBS and plasma HIV-1 viral load measurements. The quantitative level of HIV-1 viral RNA was determined using 48-paired plasma and DBS samples. The levels of agreement between paired samples measurement was graphically visualized using the Bland–Altman plot. The vertical (y-axis) and horizontal (x-axis) of the Bland-Altman plot reveal the difference and mean (average) of the two-paired measurements for each sample, respectively. A horizontal line (drawn in red) on the plot represents the mean of all the differences between the two-paired samples. The two horizontal lines (drawn in yellow) represent the lower and upper 95% confidence intervals of the mean difference, respectively

25 to 94% [27–29]. Ethiopia also strongly initiated the national HIV care and treatment guidelines, which recommended routine and targeted viral load testing to individuals living with HIV receiving ART in the year 2018 to enhance the quality of HIV services [30, 31]. Therefore, having the capability to carry out periodic viral load testing is crucial for identifying early treatment failure, which improves the success rate of prompt actions and reduces the burden of drug resistance mutations [32, 33].

In the current study, prospectively staff-collected DBS and plasma samples were analyzed using the Roche COBAS AmpliPrep/COBAS TaqMan version 2.0 assay to quantify the level of HIV-1 viral RNA among people living with HIV receiving ART [34]. Our findings demonstrated that there was a significant linear correlation between the level of HIV-1 viral load measurements from the paired DBS and plasma samples. In addition, there was a high level of agreement between the DBS and plasma sample measurements on the quantitative detection of HIV-1 RNA copies as depicted in the Bland–Altman plot (Fig. 2).

Our findings were supported by previous studies from the USA [35], Cameroon [36], and India [37, 38], which demonstrated that HIV-1 viral load measurements analyzed using the automated Abbott Real-Time viral Load assay were highly correlated, and comparable mean differences were observed between the DBS and plasma samples. In addition, the current findings were further supported by previous studies from Nigeria [39], Spain [40], Thailand [41], and Kenya [42, 43]. Which demonstrated that the quantitative HIV-1 viral load results obtained using the NucliSENS EasyQ automated system, Abbott Real-Time HIV viral load test, and Roche COBAS AmpliPrep/COBAS TaqMan assay were no significantly different and the HIV-1 viral load results from these studies were comparable across the plasma and DBS sample measurements.

Moreover, the current findings were also in line with previous studies from Tanzania [44], Mexico [45], Malawi [46, 47], Netherlands [48], and France [49]. The quantitative viral load measurements from paired DBS and plasma samples using NucliSENS EasyQ HIV-1 assay, Amplicor HIV-1 Monitor test, and Roche COBAS AmpliPrep/COBAS TaqMan assays were highly correlated and the level of agreement between DBS and plasma sample measurements were acceptable, suggesting that the DBs sample is a suitable alternative HIV-1 viral load monitoring sample source.

The WHO has planned that, by 2030, 95% of all people living with HIV will have been diagnosed, 95% will be on

life-saving ART, and 95% will have achieved a suppressed viral load to prevent HIV from spreading to others [10, 50]. In this context, high correlation and agreement between plasma and DBS samples could have great implications for enhancing HIV viral load detection coverage by using an alternative DBS sample due to the higher stability of DBS samples compared to plasma. Adopting this alternative approach could potentially enable significant reductions in the turnaround time of the diagnosis and enhance the workflow in the laboratory. Therefore, the comparable quantitative HIV-1 RNA measurements between plasma and DBS obtained in the current study suggest the scientific reliability of DBS for HIV viral load testing and provide baseline information for policymakers and healthcare providers in the region to consider integrating DBS into routine HIV care programs, potentially improving accessibility and coverage.

As a strength, this study addresses the most feasible alternative approach (DBS) for prompt HIV-1 viral load monitoring in a local context since many resource-constrained settings frequently face challenges in the accessibility of cold chains for plasma sample transportation and storage. As a limitation of the study. The sensitivity and reliability of the viral load measurements could be compromised by the substantially smaller volume of blood extracted from the DBS samples compared to plasma, particularly during extremely low viral load levels.

Conclusion and recommendations

The current study demonstrated a high correlation between the DBS and plasma HIV-1 RNA measurements, with a significant linear correlation over the quantitative range. In addition, the level of agreement between the paired DBS and plasma sample measurements was also high. Therefore, considering minimal invasive blood collection, higher stability at room temperature or ease of transportation, and a decentralized sample collection procedure. The DBS samples could be used as an alternative approach for periodic monitoring of HIV viral loads to scale up the HIV diagnosis and treatment coverage, particularly in areas with limited laboratory settings.

Abbreviations

ART	Antiretroviral Therapy
DBS	Dried Blood Spot
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
QS	Quantification Standard
RNA	Ribonucleic Acid
UoG-CSH	University of Gondar Comprehensive Specialized Hospital
WHO	World Health Organization

Supplementary Information

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Supplementary Material 1: S1 Table: Socio-demographic and clinical variables assessment form (PDF).

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Author contributions

Conceptualization: FK, GG, NB, and ML. Data curation: FK, GG, GB, MA, and TW. Formal analysis: GG, MA, and ML. Investigation: FK. Supervision: GG, GB, NB, and ML. Methodology: FK, GG, GB, MA, TW, NB, and ML. Software: GG and ML. Validation: GG and ML. Visualization: GG and ML. Writing-original draft: FK, GG, MA, TW, and ML. Writing-review & editing: GG, GB, NB, and ML. All the authors read and approved the final manuscript.

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Data availability

All relevant data are within the manuscript and its Supporting Information files.

Declarations

Ethics approval and consent to participate

The current study was performed after obtaining ethical approval from the ethics committee of the Institute of Biotechnology, University of Gondar, Gondar, Ethiopia. Written informed consent and informed assent were obtained from all study participants and parents/guardians of study participants under 18 years of age, respectively. The confidentiality of the participant's data was kept by assigning a unique code to each study participant, and the study was conducted as per the Helsinki Declaration for Biomedical Research.

Competing interests

The authors declare no competing interests.

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