Research Article

Assessment of the accuracy of dried blood spot (DBS) sample in HIV-1 viral load as compared to plasma sample using Abbot assay

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ABSTRACT

Background: HIV has claimed millions of lives with the Sub-Saharan Africa being the most affected. There is a significant increase in access to antiretroviral drugs which also demands frequent monitoring to determine the drug effectiveness and efficacy. Thus there is a great need to evaluate simplified methods to monitor treatment with such antiretroviral drugs. Use of dried blood spots (DBS) can be ideal if evaluated in resource limited countries such as Malawi since they are easy to collect, store and convenient. The main objective of this study was to evaluate the accuracy of a dry blood spot sample in the quantification of viral particles in HIV reactive patients using the Abbott m2000rt assay.
Methods: 87 participants were recruited from the ART clinic at Queen Elizabeth Central Hospital using convenience sampling method. 29 were on antiretroviral therapy and 58 had not started the therapy. HIV-1 RNA extraction and quantification was performed from DBS and plasma using Abbott m2000sp and m2000rt systems respectively. The results were statistically analyzed by Bland-Altman method using medcalc software version 12.6.1.
Results: 66 paired samples with detectable viral loads were analysed. These gave a correlation of 0.98. The mean difference was 0.05 $\log_{10}$ copies/ml with a standard deviation of 0.17 at 95% confidence interval.The Bland-Altman plots showed limits of agreement which ranged from -0.38 to 0.28 log10 copies/ml at 95% confidence interval.
Conclusion: Results showed strong agreement between the plasma and DBS samples. A slight and clinically insignificant difference was observed between the two methods. A larger sample size can give support to the study findings. Since samples were less than a week old, it is not known if the results would be different if they were to be stored for a longer period.

Keywords: Viral load, Dry Blood Spot (DBS), HIV, Accuracy, Plasma, Malawi

INTRODUCTION

According to 2012 UNAIDS global report, 34 million people were living with HIV in 2011.Sub-saharan countries are the most affected with 69% of the people living with the virus globally. By the end of 2011, 8 million people were receiving antiretroviral treatment for HIV/AIDS globally, of which 6million is in the sub-Saharan region. An increase of 63% of the people accessing ART was seen between the year 2009 and 2011.[1] Diagnosis of HIV using nucleic acid amplification testing (NAT) has become a routine in resource constrained settings, in order to initiate ART programs, care and treatment programs.[2,3] Up to now it is very difficult to predict or recognize virological failure by clinical or immunological monitoring among patients taking ARVs.[4,5] Low and middle income countries has shown great interest in using NAT services to monitor plasma HIV-1 RNA levels to those patients on ART.[3,4]

In industrialized countries, monitoring of treatment with viral load measurements and drug resistance testing is the standard of care to ensure early detection of treatment failure and a prompt switch to a fully active second-line regimen, before drug-resistant mutations accumulate. This is also recommended by WHO. These tests, however, require highly specialized laboratories and stringent procedures for storage and shipment of plasma, and are rarely available in resource-limited settings. Therefore, treatment failure in such settings is usually not detected until patients develop severe immunodeficiency, at which stage widespread resistance is likely.[6] There is an urgent need to evaluate simplified methods to monitor treatment efficacy and to guide an appropriate switch to second line therapy. Evidence has been accumulating that identification of treatment failure based only on clinical or immunological criteria can be unsatisfactory because of a late identification of virological failure with consequent accumulation of resistance mutations.[7,8] Dried blood spots (DBS) are easy to collect and store, and can be a convenient alternative to plasma in settings with limited laboratory capacity.[6]

Plasma is considered to be the most appropriate specimen type for HIV-DR-genotyping. However, use of plasma may not be feasible in rural, remote areas in limited resource settings (RLS), since its preparation and storage requires personnel and laboratory infrastructure that is often lacking. An alternative specimen type for HIV-DR genotyping are dried blood spots (DBS). DBS can be made from blood drawn for routine clinical or surveillance purposes without special laboratoryprocessing.[2,6] The filter paper used is easily obtained and stored, and although procedures for making DBS must be followed precisely, the training required is less intensive than that required for plasma separation. The viral RNA in DBS is stable over longer periods and freezing is not required unless storage over one month is planned. Finally, in RLS, DBS are more easily transported than plasma to the accredited drug resistance testing laboratory because they can be shipped as non-hazardous materials using regular mail or courier services.[9]

METHODS

87 participants were recruited from the ART clinic at Queen Elizabeth Central Hospital in Blantyre Malawi using convenience sampling method. Out of the 87 participants, 29 were on antiretroviral therapy and 58 had not started the therapy. 5ml of blood from HIV patients was drawn using a 10ml fitted syringe. The blood was then transferred into a 5ml Ethylene-diamine-tetra-acetic acid (EDTA) collecting tube. The rest of the blood in the tubes was centrifuged and plasma separated and transferred into micro vials labeled with corresponding patient's study identity. The samples in the micro vials were stored at -80°C before processing and the DBS cards were stored at room temperature in plastic bags.

Plasma sample preparation: Plasma samples were removed from the freezer and allowed to thaw at room temperature. 1ml of each sample was transferred into the m2000sp reaction vessels labeled with the patient's ID number. These samples were put into the instrument sample rack together with three controls, (lot number: 4412214) and 6 calibrators' (lot number: 436449) from Abbott reagent manufacturers in USA.[10]

DBS sample preparation: Under a safety cabinet a sterile puncher was used to punch two spots from each DBS card into a 50ml voltex tube. After punching all the samples into their tubes, 2ml of lyses buffer provided with the Abbott reagents was added in each tube. The samples were incubated at room temperature for 30 minutes with intermitted mixing and the content were transferred into the m2000sp reaction vessels labeled with the participant's study number. The lysate in the reaction vessels was placed in the sample rack together with 3 controls and put in the m2000sp instrument for RNA extraction.

Plasma nucleic acid extraction: The 0.6ml HIV-1 RNA extraction protocol installed in the machine was used for extraction of the nucleic acid from the plasma. Internal controls were included as well as the human plasma negative control, low positive control and a high positive control.

RNA extraction was performing following the machine protocol (the automated m2000sp instrument) and reagents manufacturer's instructions. After extraction, the RNA extracts from the sample were mixed with the amplification reagents in a dip well plate provided with the Abbott reagent kit. The reaction mixture was then dispensed into a 96 well optical reaction plate and was ready for amplification.

DBS nucleic acid extraction: The DBS extraction was done following the analyser protocol. 1.0ml HIV-1 RNA protocol installed in the machine was used for RNA extraction. The m2000sp then mixed the RNA extracts with the amplification reagents and dispensed the reaction mixture into the Abbott 96 well optical reaction plate.

Plasma sample amplification: The 96 well optical reaction plate from the m2000sp machine was transferred into the m2000rt real time PCR machine for RNA amplification. 0.6ml HIV-1 RNA protocol to amplify plasma RNA was used.The amount of HIV-1 target sequence that is present at each amplification cycle was quantified by measuring the fluorescence of the HIV-1 probe that binds to the target during the extension/anneal step. The 0.6mL HIV-1 RNA protocol was used in accordance with the manufacturer's instructions for the plasma samples. Fluorescence counts were converted directly into viral load measures by the m2000rt analyser.[11] The results were printed and ripped into a CD for further analysis.

DBS sample amplification: The two 96 well optical reaction plates, (each containing 48 samples from the DBS extraction) were transferred into the m2000rt instrument

for RNA amplification. The 1.0ml DBS HIV-1 RNA protocol was not installed in the machine, so the 0.6ml HIV-1 RNA protocol was instead used to amplify the RNA. The protocol that was missing (1.0mL DBS HIV-1 RNA) enables the instrument to convert the fluorescence counts directly into viral load automatically and is under experiment. The measurements obtained by the PCR machine were printed and ripped into a CD for further analysis. A correction factor based on the different volumes input was used to correct the results obtained from the m2000rt instrument. After the correction of the DBS results, they were paired with the plasma results obtained by the same machine and transferred to excel. The paired results in excel were then $\log_{10}$ transformed.

RESULTS

Out of the 96 DBS samples assayed, 5 DBS samples produced invalid results because they did not give any result. This could be due to insufficient sample, bubbles in the sample among other reasons. 66 samples gave detectable values and they were analysed. The correlation between the viral load values obtained from the 66 paired sample was high with a correlation coefficient of 0.98.

Table 1: HIV-1 RNA detection rates in paired DBS and plasma samples.

Viral load in $\log_{10}$ copies/mL	Plasma samples	DBS samples	Detection rate
<2.6 (not detected) (<400copies/ml)	25	25	100%
>2.6 – 3.0 (>400-1000copies/ml)	5	5	100%
>3.0- 3.7 (>400-1000copies/ml)	3	1	33.3%
>3.7- 4.0 (>5000-10000copies/ml)	5	5	100%
>4.0 (>10000 copies/ml)	53	55	103.8%

Viral load measurements of 91 samples. DBS sample measurement in the >3.0-3.7 range a detection rate of 33.3% and those >4.0 had a detection rate of 103.8% with DBS detecting n=55 instead of 53.

40(44%) DBS samples had higher viral load values with the difference ranging between 0.01-0.56 $\log_{10}$ copies /ml from the plasma samples, while 26(29%) of the DBS samples had lower viral load values ranging from 0.01-0.44 $\log_{10}$ copies/ml from the plasma ones. The viral load

in 25(27.8%) DBS samples matched those of plasma viral load with no difference. The mean difference obtained was 0.05 $\log_{10}$ copies/ml for the 66 paired samples with detectable viral load measurements after Bland-Altman plot with a standard deviation of 0.17 at 95% confidence interval. The limits of agreement ranged from -0.38 to 0.28 $\log_{10}$ copies/ml at 95% confidence interval. Bland-Altman plots for samples with measurements >3.7 $\log_{10}$ copies/ml (~5,000 copies/ml) (n=60) which are considered clinically significant showed a good agreement.[12] The mean difference was at 0.055 $\log_{10}$ copies/ml with a standard deviation of 0.172 at 95% confidence Interval. The limits of agreement ranged from -0.39 to 0.28 $\log_{10}$ copies.
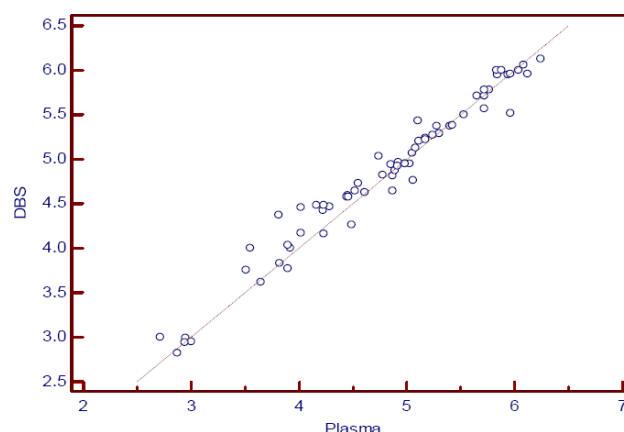

Figure 1: Correlation between plasma and DBS.

Correlation between HIV-1 RNA levels measured with the Abbott Real-Time assay in DBS (y-axis) and plasma (x-axis) samples. Each data point represents one of the 66 samples assayed.
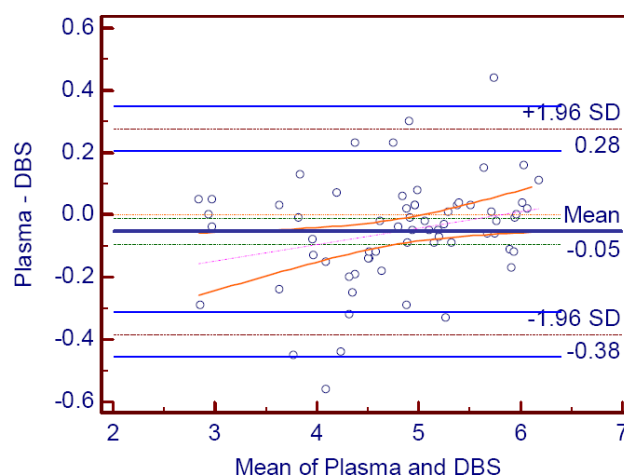

Figure 2: Agreement between plasma and DBS.

Bland-Altman analysis of agreement between plasma and DBS with regression line of the differences, n=66

On the plasma results, 5 of the results were invalid, therefore removed from the study, which brought the sample number to 91 including controls and calibrators results. The agreement of the results was assessed using

the Bland-Altman method incorporated in MedCalc version 12.6.1.

DISCUSSION

In this study, the accuracy of a dried blood spot sample in the quantification of viral particles in HIV reactive patients using the Abbott m2000rt assay was evaluated. The viral load result of DBS and plasma (n=66) showed a good correlation (r=0.98). The study also showed a very good sensitivity of DBS of 94.8% in general. The DBS results in the 66 samples showed minimal differences (0.05 log_{10} copies/ml) from those of plasma, which is not clinically significant. 44% (n=40) of the 66 samples showed a difference ranging between 0.01- 0.56 log_{10} copies/ ml from the plasma samples. 29% (n=22) of the 66 DBS samples showed lower viral load values with differences ranging from 0.01-0.44 log_{10} copies/ml from those of plasma. 27.8% (n=25) of the 66 samples were undetectable. Altman-Bland plot of the 66 paired samples showed a mean difference of 0.05 log_{10} copies/ml, SD=0.17 at 95% confidence interval, which is not clinically significant. The Bland-Altman plot also showed clinically acceptable limits of agreement ranging from -0.38log_{10} copies/ml to 0.28log_{10} copies/ml at 95% confidence interval. Bland- Altman plot for DBS samples with viral load >3.7log copies /ml (n=60) showed a good agreement with mean difference of 0.05 log_{10} copies / ml, SD= 0.172, 95% CI. Viral load measurement of >3.7log_{10} copies /ml is considered clinically significant hence the Bland –Altman plot showed acceptable limits of agreement in the range of -0.39log_{10} copies/ml to 0.28 log_{10} copies /ml. The overall detection rate of the DBS was high. However this study revealed a slightly higher detection rate of 103.8% in some results in the DBS sample (>4.0 log copies/ml). This could be due to prodigal DNA present in the peripheral blood mononuclear cells which leads to a falsely elevated viral load measurement.[12]

Several studies have shown that DBS is a practical alternative to plasma for quantifying HIV-1 viral load.[11, 13, 14] In resource limited countries it is not easy to store and transport samples because of the cold conditions required and collection techniques. Therefore this becomes interesting knowledge[14]. The good agreement between the DBS and plasma methods with minimal mean difference (bias), the high sensitivity and specificity shown in the study is in agreement with other several study findings.

CONCLUSION

The study had shown that DBS sample is comparable to plasma sample, hence could be used alternatively or replace the plasma sample in monitoring treatment failure and used in resource limited settings such as Malawi. Using DBS as a sample could reduce cost in terms of storage, since it is stored at room temperature as well as reduce transportation cost since it does not need

maintenance of cold chain through-out transportation. False results due to contamination could be minimised, as it is kept in dry forms before analysis.

The findings call for further investigations to provide a comparative analysis. The sample size in this study was not enough to draw such conclusions hence the need for a high sample size in different set-ups. On the other hand, the DBS sample used were stored for a short period of time (all less than a week old). There is need to test DBS samples stored at different intervals (i.e. after 1/2/3 or 4 weeks/ months) to observe if they would still be valid.

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