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RESEARCH ARTICLE

IMPLEMENTING HIV VIRAL LOAD TESTING FOR MONITORING ANTIRETROVIRAL THERAPY IN COTONOU, BENIN: PRELIMINARY REPORT

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ABSTRACT

In low resource - countries like Benin, HIV viral load is rarely available for monitoring antiretroviral treatment, which is generally based in these settings on clinical assessment together with T-cell CD4 count. In this study, we present our experience of setting up HIV viral load in Cotonou and the relative contribution of this test in monitoring such patients. From May 2013 to January 2014, 139 participants aged above 15 years were tested for both T-cell CD4 count and HIV viral load. We observed that HIV viral load was above 3 Log_{10} copies/ml in 15 participants (10.8%) though their CD4 count was above 350 cells/µl. HIV viral load could help for early detection of patients with incomplete virologic response to antiretroviral treatment in Cotonou.

INTRODUCTION

In 2012, 9.7 million people in low and middle - income countries received antiretroviral therapy (ART), representing 61% of all those who were eligible under the World Health Organization (WHO) HIV treatment guidelines (World Health Organization, 2010; UNAIDS, 2013). Unfortunately, despite the increased availability of highly active antiretroviral therapy (HAART), there was no improvement in the monitoring of such patients in these countries. Indeed, though it has been documented that virologic failure precedes immunologic failure, which in turn precedes clinical failure in patients under HAART, viral load (VL) monitoring is not routinely performed in resource-limited settings (Rawizza HE *et al*., 2011). In contrast, VL monitoring is considered as the gold standard for assessing treatment success in developed world (Thompson *et al*., 2010; Clumeck *et al*., 2011). In this study, we present a preliminary report on implementation of HIV VL in monitoring patients on HAART in Cotonou, Benin using NucliSENSEasyQ equipment (bioMerieux, France) and discuss challenges in setting up such technology in low - resource countries.

MATERIALS AND METHODS

Steps in setting up VL in the laboratory.

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Installation. Prior to implementation of VL, the laboratory was renovated to include a molecular biology unit which comprised of 3 different cubicles, each dedicated for pre PCR (mix preparation), nucleic acid extraction, and amplification. A new NucliSENSEasyQ equipment was installed by a local supplier while laboratory personnel such as biologists and technicians with good experience in molecular biology were trained on the use of the device. *Comparison with Abbott m2000rt.* Summary comparison was performed on 7 different samples between the new machine (NucliSENSEasyQ) and another VL equipment, namely Abbott m2000rt (Abbott Molecular, USA). Abbott m2000rt was available in a reference laboratory and samples for comparison were collected from HIV positive and HIV negative participants. *Positive controls.* Apart from the internal control, blood from HIV positive patient identified during a blood donation campaign was used as positive control since there was no positive control in NucliSENSEasyQ kit. The plasma was separated from the rest of the blood, tested with both NucliSENSEasyQ and Abbott kits, and stored in several aliquots at -20°C for routine use as positive control.

Routine assessment of patients using VL and T- cell CD4 count

From May 2013 to January 2014, all consenting participants aged 15 years and above who were receiving HAART for more than 6 months were recruited into the study. For each participant, T-cell CD4 count was performed using the CyFlow counter (Partec, Allemagne) while HIV VL was measured by the NucliSENSEasyQ HIV-1 machine. For both tests, positive

and negative quality controls were performed. For T-cell CD4 count, 50 µl of EDTA-anticoagulated blood were added to 10 µl of monoclonal antibodies. After 15 min of incubation, 1 ml of dilution buffer was added and the sample tube was attached to the CyFlow Counter for automated counting. Results were expressed in a histogram (CD4+cells/µl). For nucleic acid quantification, amplification and detection were performed by adding a mixture of reagents (primers solution, molecular beacon probes, nucleotides, dithiothreitol, KCl, MgCl2) to a purified nucleic acid extract. The mixture was incubated in a NucliSENSEasyQ Incubator, and the enzyme solution added to each tube. The sealed tubes were then transferred to the NucliSENSEasyQAnalyzer. Results were expressed as valid (positive or negative) or invalid. Viral load was calculated (Log_{10} copies/ ml) for each positive result.

RESULTS

After renovation of the laboratory and acquiring the NucliSENSEasyQ equipment, consumables and reagents, it took about 4 weeks to complete the whole process of setting up VL in the laboratory. This comprised of installation of the equipment, training by the supplier, summary comparison with Abbott equipment and starting a routine testing of VL. Results summary comparison between NucliSENSEasyQ and Abbott are presented in Table 1.

Table 1. Summary comparison between Abbott m2000rt and NucliSENSEasyQ

No of Sample	Viral load (Log_{10} copies/ml)	
	Nuclisens	Abbott
1	4.7	5.7
2	4.2	5.3
3	7.0	6.8
4	3.9	3.8
5	< 1.7	< 1.7
6	< 1.7	1.9
7	< 1.7	2.1

Apart from two samples (samples 6 and 7) with low VL with Abbott but undetectable results with NucliSENSEasyQ, there was an agreement between the two techniques for other samples. Of the 139 participants included in the study, 82 (59.0%) were males while 57 (41.0%) were females. One hundred and twenty three of them (88.5%) were under first - line drugs regimen and 16 (11.5%) were receiving second - line drugs. Concerning T-cell CD4 count, 54 (38.9%) of the participants had their CD4 count less than 200 cells/ µl, 26 (20.1%) of them had 200 – 350 cells/ µl while 57 (41%) had above 350 cells/ µl. Although, VL testing can be carried out for both adult and paediatric patients under HAART, results from only adult patients are presented in this study. VL was found to be less than 1.7 Log_{10} copies/ ml in 60 participants accounting for 43.2%, 1.8 - 3 Log_{10} copies/ ml in 17 participants (12.2%), 3 - 5 Log_{10} copies/ ml in 32 participants (23.0%) while ≥ 5 Log_{10} copies/ml was seen in 30 participants (21.6%). In total, VL above 3 Log_{10} copies/ml was seen in 15 participants (10.8%) who had CD4 count above 350 cells/µl (Table 2). There were 5% invalid results that became either negative or positive valid result after repeated testing.

Table 2. HIV viral load and T-cell CD4+ count of participants

Viral Load (Log_{10} copies/ml)	T-cell CD4 count (cells/µl)						Total	
	<200		[200-350]		>350			
	No	*%*	No	*%*	No	*%*	No	*%*
≤ 1.7	12	*22.2*	13	*46.3*	35	*61.4*	60	*43.2*
[1.8-3[	05	*09.2*	05	*17.9*	07	*12.3*	17	*12.2*
[3 - 5[	16	*29.6*	05	*17.9*	11	*19.3*	32	*23.0*
≥ 5	21	*39.0*	05	*17.9*	04	*07.0*	30	*21.6*
Total	54	*100*	28	*100*	57	*100*	139	*100*

DISCUSSION

Several technologies are available to measure HIV VL ((Choi *et al*., 2009; Gomes *et al*., 2013). Of these methods, isotherm amplification technology, used by NucliSENSEasyQ has been found more suitable for low - resource settings as it is simpler to use, easier to implement with rapid turnaround time (4 h), particularly the extraction step, than other commercialized plasma RNA quantification kits (Kébé *et al*., 2011). The rapid implementation of this technique in our laboratory was favoured by the fact that the staff available had an extensive experience in molecular biology. However, one of the major limitations of the technique is the absence of positive control in the kit package. To overcome this problem, we used plasma from an HIV positive patient found during blood donation. The sample was then aliquoted, tested by the two techniques and used in each round of tests along with routine samples. Five percent of the samples tested were invalid; this was resolved by repeating the tests. Possible explanations of invalid results are suboptimal RNA extraction steps, and/or errors in pipetting extracts and reagents because very small quantities were used. VL was > 3 Log_{10} copies/ml in 15 patients (10.8%) who had T-cell CD4 count above 350 cells/µl (Table 2). These patients with incomplete virologic suppression but high T-cell CD4 count, may either have a treatment failure or poor treatment compliance (Sollis *et al*., 2014). In such situations, clinical management of patients should be reviewed early enough in order to prevent development of opportunistic infections and death. Even though, VL is expensive, it could therefore be cost-effective by reducing morbidity and mortality in HIV patients under HAART. This study showed that in our setting, using only clinical and immunological parameters to monitor HIV patients under HAART is not optimal. So far, routine implementation of VL in low - resource countries has been limited by the cost of the equipment as well as the complexity of setting up the technologies (Rouet and Rouzioux, 2007; Rouet *et al*., 2008 ; Arnedo *et al*., 2012). Another constraint is a continuous supply of consumables and reagents. In our case, initial supply was facilitated by an International Non-Governmental Organization (NGO) before the National AIDS Programme took over the supply of consumables and reagents. Maintenance of equipment can also be a bottleneck to

implement new technology. After the initial training, technicians were able to perform routine maintenance but a periodic maintenance was contracted with the supplier. Furthermore, a permanent and reliable power supply is also an issue for implementing a molecular biology laboratory. This was taken into account by connecting the laboratory to a back-up generator and an installed central uninterrupted power supply (UPS).

Several studies have been performed on comparison between NucliSENSEasyQ and Abbott m2000rt using either dried blood spots or plasma samples with generally good agreement (Choi JY *et al*., 2009; Gomes *et al*., 2013). Recently, NucliSENSEasyQ has been shown to perform well when compared with other equipment such as Amplicor Roche (Xu *et al*., 2012; Sollis*et al*., 2014). From our study, we found 2 discordant results from two samples with low VL with Abbott but undetectable with NucliSENSEasyQ (Table 1). Discordant results may be explained by the fact that in very low positive samples, positivity of tests is found by chance according the Poisson's binomial distribution; nevertheless a slight superiority of Abbott technique cannot be excluded (Vermeulen *et al*., 2013). This discordance however, has practically little impact in monitoring patients since no change in the management of patients is indicated if such low VLs are found during follow-up.In conclusion, implementing VL for monitoring HAART was feasible in Cotonou and the test may help in early identification of patients with risk of treatment failure.

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