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QUANTIFICATION OF HUMAN IMMUNODEFICIENCY VIRUS -1 VIRAL LOAD USING NUCLEIC ACID SEQUENCE-BASED AMPLIFICATION (NASBA) IN NORTH CENTRAL NIGERIA

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ABSTRACT

Background: Viral load (VL) quantification is considered an integral part of the standard care in human immunodeficiency virus (HIV) infected individuals but in Nigeria as in most of sub-Saharan Africa, this has not reached the majority of patients.

Methods: We report the first field application of the NucliSens EasyQ HIV-1 platform for the real time quantification of HIV-1 VL combining NASBA amplification and real time detection with molecular beacons among HIV-1 infected individuals in north central Nigeria where the predominant HIV-1 subtypes are CRF02_AG and G. $CD4^+$ counts were enumerated using a fluorescence-activated cell sorter system.

Results: Of one hundred and forty nine (n=149) plasma sample from patients with mean age of 32 years and made up of 77 males and 72 females, fifty {n = 50 (37.9%); 28 males and 22 females}had VLs below the lower detection limit (LDL=25 IU/ml) set by the assay while eighty- two {n = 82 (62.1%); 39 males and 43 females}had VL levels above the LDL. Furthermore, 13 of 82 (15.9%) patients with viral loads above the LDL had VLs between 26-1000 IU/ml while 69 (84.1%) had VLs of 1001-2400000 IU/ml. 17 (11.4%) of the samples could not be analyzed due to poor viral amplification. Among individuals with both $CD4^+$ and VL results (n=56), those with $CD4^+$ of 1-418 cell/µl presented with higher VL usually above 45,000 IU/ml when compared with those with $CD4^+$ of over 500 cell/µl.

Conclusion: Our findings highlight the pattern, usefulness and feasibility of VL quantification by NucliSens EasyQ in monitoring HIV-1 patients in Nigeria.

Key Words: HIV-1, Viral load quantitation, Nigeria *(Accepted 23 July 2009)*

INTRODUCTION

Nigeria has the third highest burden of the human immunodeficiency virus (HIV) infection in the world, with people living with HIV estimated to total over 4.0 million (1). In 2002, the implementation of one of Africa's largest antiretroviral (ARV) treatment programmes was commenced in Nigeria (2). A standard, functional and effective laboratory monitoring system must be put in place for ARV-treatment programs to be successful. Many clinical and laboratory markers including circulating $CD4^+$ and $CD8^+$ T cell counts, p24 antigen and ß-2 microglobulin quantification have been used for determining the prognosis in HIV-1 infection (3, 4). Direct HIV-1 RNA quantification has recently become a useful method for assessing and monitoring HIV-1 disease progression. Viral RNA in plasma directly reflects the titer of HIV-1 during infection and is a better predictor of subsequent risk of AIDS or death than the other markers cited above (4). As a direct measurement of circulating virus, HIV-1 viral load is expected to give complementary information to other markers that monitor the immunological status, as lymphocytes subsets cell counts and seric ß-2 microglobulin levels. Besides, viral load (VL) has enabled physicians to monitor the efficacy of antiretroviral therapies since it has been shown that treatment failures correlates with a rapid and dramatic decrease in the HIV-1 RNA level and vice versa (5).

Tremendous strides have been made in treating and monitoring HIV-1 infection. Combination antiretroviral (ARV) drugs therapy suppresses virus replication, delays disease progression, and reduces mortality (5, 6). Plasma VL assays are used in combination with $CD4^+$ cell counts to determine when to initiate therapy and detect when a regimen is failing. Recently, the United States Department of Health and Human Services guidelines advocate the use of plasma viral load testing when considering ART initiation, monitoring response to therapy, and instituting a change in drug regimen (6). Unfortunately, the full benefits of antiretroviral drugs and monitoring tests have not yet reached the majority of HIV infected patients who live in sub-Sahara Africa where the impact of the disease is greatest and where differences in the HIV-1 genotypes could introduce a bias in the assay (1). Numerous countries in sub-Saharan Africa have

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elected to base their treatment follow-up on CD4+ monitoring alone which is known to be inherently inconsistent and could be misleading (7, 8).

We report the results of the first field performance and application of the NucliSens EasyQ viral load assays for the quantitative detection of HIV-1 RNA in patients infected with HIV-1 in Nigeria where the predominant subtypes are CRF02_AG and G (9). The relationship between $Cd4^+$ T-cells and viral load was also assessed.

METHODOLOGY

Study population: One hundred and forty nine (n=149) blood samples were collected by venepuncture from patients attending our HIV treatment program at two Innovative Biotech (IBL) sites in North Central Nigeria (IBL clinic- Keffi and IBL clinic- Abuja- North central Nigeria) as part of their treatment monitoring between March 2006 and October 2007. Five-milliliter EDTA blood samples were collected and centrifuged, and the plasma was used for routine HIV-1 VL analysis. In our facility, VL measurement is considered mandatory for HIV monitoring. The plasma samples were placed in NucliSENS lysis buffer after collection and prior to testing to preserve the nucleic acid of the virus. Only HIV-1 positive patients were considered for this study. Individuals with HIV-2 or dual infection were excluded from the study. The study was cross-sectional in design.

Viral load determination: The NucliSENS miniMAG platform version 1.0 (revision 1) for nucleic acid extraction and NucliSENS EasyQ HIV type 1 (HIV-1) analyzer version 2.0 (revision 0) (BioMérieux , Boxtel, The Netherlands) were used for the real time quantification of viral load in plasma samples by combining NASBA amplification with molecular beacon (MB) detection technology. NASBA amplification primers and MB detection probes were directed towards a conserved region of the HIV-1 genome. RNA extraction from plasma samples was performed with a silica-based procedure using the NucliSens extractor. Subsequently, NASBA amplification and real-time detection of HIV-1 RNA and internal calibrator RNA was performed in a single tube using a temperature-controlled fluorescence reader. Quantitative results were calculated using a curve fitting of signal curves for both the HIV-1 RNA and an internal calibrator. In a single run, test results were obtained in 90 minutes with less than 30 minutes hands-on time. All assays were conducted according to the manufacturer's instructions and good laboratory practice standards. Further details of the procedure are as described by Weusten et al, 2002 and Stevens et al, 2005 (10, 11). All values were reported according to the limits (linear range: 25 IU/ml to 3,000,000 IU/ml) set by the EasyQ assay. Statistical analyses were performed on the results of samples with valid NucliSENS EasyQ HIV-1 results.

$CD4^+$ enumeration: The $CD4^+$ values for fifty six (n = 56) of the HIV patients with known viral load were also enumerated using our previous method (12). Briefly, we used a superior fluorescence-activated cell sorter system (FACScount, Becton Dickenson FACScount, Canada) according to manufacturer's instructions. This system quantifies $CD4^+$ T lymphocytes as absolute numbers of lymphocytes per L of blood and results recorded in an automated fashion. The $CD4^+$ and VL were compared using chi-Square test at 95% confidence level. All the participants in this study signed informed consent form after the purpose of the study was explained to them by our counselors and the study protocol was approved by the research committee of Innovative Biotech.

RESULTS

A total of 149 clinical samples from patients aged between 5 and 59 years (mean = 32 years) and made of 77 males and 72 females respectively were available for HIV-1 viral load quantitation. Seventeen (n = 17; 11.4%; 10 males and 7 females) samples could not be included in the analysis because of invalid EasyQ results arising from poor viral RNA amplification. The results of the remaining 0ne hundred and thirty two (n = 132) samples were grouped into two: Group 1, had viral loads below detection levels (LDL = 25 IU/ml) while group 2 were those above detection limit (below the detection limit is designated LDL by the analyzer). Fifty {n = 50 (37.9%); 28 males and 22 females} had viral loads below the detection limit set by the assay while eighty- two {n = 82 (62.1%); 39 males and 43 females} had viral loads above the detection limit set by the assay. Furthermore, 13 of 82 (15.9%) patients in group 2 had viral loads between 26 and 1000 IU/ml while 69 (84.1%) had viral loads of 1001-2400000 IU/ml. 10 of 132 (13.2%) patients had HIV-1 viral load of 50000-2400000IU/ml. Of the 56 individuals with both $CD4^+$ and viral load results, those with $CD4^+$ values of 1 to 418 cells/µl presented with higher viral load values of 45,000 IU/ml and above when compared with those with $CD4^+$ values above 500 cells/µl were VLs tended to be nearer the LDL set by the assay.

DISCUSSION

It is clear that a highly active antiretroviral treatment (HAART) is able to reduce viral load and partially repair the immunological damage caused by HIV-1 infection (13). VL is therefore an important marker of HIV prognosis and there is a general acceptance among scientists that viral load monitoring is the best

measure of HIV/AIDS disease progression (6, 8). The NucliSens EasyQ assay has been evaluated in South Africa and China for the quantification of HIV-1 VL where the predominant subtype is C and have been found to be very reliable, effective ,sensitive, specific and reproducible (11,14,15). This instrument automates the labor-intensive washing steps typical of nuclic acid extraction, thereby significantly reducing hands-on time.The NucliSENS miniMAG-NucliSENS EasyQ HIV-1 combination provides a feasible platform for HIV viral load quantification. The gains of this technology has been reaped by industrialized countries while countries in sub-Sahara Africa keep relying on the inherently inconsistent $CD4^+$ counts as a first choice marker in HIV/AIDS monitoring (8, 10). In Nigeria, to the best of our knowledge no study has been published describing the use of the NucliSENS EasyQ real time boom technology in HIV-1 VL monitoring. We demonstrate that the NucliSENS EasyQ HIV-1 with its very wide linear range (25 IU/ml to 3,000,000 IU/ml) is usable Nigeria. One of the reasons advanced against the use of the VL test in Nigeria is cost. The NucliSENS EasyQ is known to be more affordable compared to the Roche Amplicor (11) and the manufacturers are decreasing kit costs and in some instances equipment costs for resource-limited countries, thereby making this more affordable.

As a result of invalid EasyQ results due to poor viral RNA amplification, 11.4% of the plasma samples could not be included in the final analysis. In a related study, Weusten et al., 2002 (10), had recorded that 16.6% of 218 clinical samples could not be analyzed for various reasons. 37.9% of our patients (28 males and 22 females}had VLs below the detection limit set by the assay. Evidence shows that keeping the VL levels as low as possible for as long as possible decreases the complications of HIV disease and prolongs life (16). Therefore, the goal of anti-HIV treatment is to keep VL for as long as possible below the limit of what the VL test can detect. Interestingly, 46 of the 50 patients with LDL were on ART (lamivudine 150mg, stavudine 30-40mg and niverapine 200mg) that is known to be able to reduce VL. The treatment history of the remaining 4 patients with LDL is unknown to us. On the other hand, 62.1% of our patients (39 males and 43 females}had viral load values above the LDL. These individuals are at risk of getting sicker and progressing faster to AIDS. Furthermore, 15.9% patients in group 2 had viral loads between 26 and 1000 IU/ml while 84.1% had VLs of 1001-2300000 IU/ml. We also report that 10 of 132 (13.2%) of our patients had viral RNA of 50000-2400000IU/ml. Individuals with a lesser or undetectable VL have lower chances of experiencing any disease progression while those with very high VL have higher chances of disease progression. The age range of the participants in this study was between 5 and 59 years (mean = 32 years).The performance of the Nuclisens EasyQ HIV-1 assay has been assessed in individuals infected with HIV non-B subtypes that are predominant in Africa and found to be very reliable with a wider dynamic range than Amplicor (17). The Roche Amplicor method is commonly used in Nigeria. Previous studies have shown that the NucliSens EasyQ assay correlated significantly with the Roche Amplicor and it's a more affordable option (11). The standard Roche Amplicor assay has a linear range of 400 to 750,000 HIV RNA copies/ml while that of the NucliSENS EasyQ is 25 to 3,000,000 IU/ml. In the NucliSENS EasyQ assay, the potential for contamination has been dramatically reduced by using the automated extraction system. This assay thus presents a real and superior option for monitoring HIV load in Africa where the burden of the disease is highest and in Nigeria where the predominant HIV subtypes are CRF02_AG and G (2, 9). In South Africa, HIV VL monitoring is used to determine the need for changing to second-line drug regimens (18). In this study, we observed that HIV-1 infected individuals with $CD4^+$ values of 1-to-418 cells/µl presented with significantly higher ($p<0.05$) viral load values usually above 45,000 IU/ml when compared with patients having $CD4^+$ values of >500 cells/µl who tended to have VLs nearer the lower detection limit set by the assay. This agrees with Olsen et al (19) who found that HIV infected individuals with $CD4^+$ count =350 cells/µl were at increased risk of AIDS or death than those with higher $CD4^+$ counts. It is worth noting that an approximately inverse relationship of VL to the $CD4^+$ T-cell count and survival time had been observed in around 80% of HIV infected individuals (20, 21).

We belief that NucliSENS EasyQ assay with respect to sensitivity, specificity, precision, reproducibility, dynamic range and linearity be recommended for use in Nigeria where the predominant HIV subtypes are CRF02_AG and G (9) and where the burden of the disease is on the increase for treatment to be meaningful. This could be used in conjunction with the already widely used and available $CD4^+$ measurement.

CONCLUSION

The real-time NucliSens EasyQ HIV-1 viral load assay meets the performance requirements for HIV-1 viral load monitoring in an HIV non-B subtype crescent like Nigeria. This paper represents to the best of our knowledge the first formal description of the application of the miniMAG-EasyQ-NucliSENS EasyQ assay in HIV viral load quantification in Nigeria. Its implemented on a broader scale in Nigeria is recommended to enable HIV infected individuals access the gold standard for routine HIV monitoring.

Competing Interests: No member of the writing team owned stock or was employed by the kit manufacturers mentioned in this article. Some free kits were provided by the manufacturers for training purposes.

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