ORIGINAL ARTICLE

Low-level of HIV-1 Seroreversion among People on Successful Antiretroviral Therapy in Cameroon: Implications for Clinical Monitoring in Resource-limited Settings

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ABSTRACT

Background: Initiating early HIV treatment results to sustained viral suppression, reduced viral reservoirs and prompt immune reconstitution that may lead to HIV seronegativity (seroreversion). Seroreversion can be misinterpreted, leading to inappropriate clinical considerations. We thus sought to determine the HIV seroreversion among antiretroviral therapy (ART)-experienced Cameroonians.

Method: A laboratory-based cross-sectional study was conducted among ART-experienced individuals with undetectable plasma viral load (less than 40 copies/mL) in 2019 at the Chantal BIYA International Reference Centre in Yaoundé-Cameroon. On all blood samples, HIV antibody testing was performed using two rapid diagnostic tests (RDTs), followed by enzyme-linked immunosorbent assay. On non-reactive samples, proviral DNA was tested on on dried blood spots (DBS) specimens.

Results: Of the 546 participants on ART (median ART duration : 5 years) and all experiencing a successful ART (VL<40 copies/ml), only 01% (5/546) had shown HIV negative results. Of these five non-reactive cases, only one case (0.18%) was non-reactive to HIV RDTs but reactive to ELISA, and four cases (0.72%) were non-reactive to both RDTs and ELISA. These four samples were also negative for HIV proviral DNA, indicating potential absence of infection or an optimal control of viral replication.

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Conclusion: Seroreversion of HIV-1 infection is possible but may occur rarely among HIV-infected Cameroonians who are on successful ART. The few cases of HIV negativity on serology and DBS-PCR (proviral DNA) underscores the need for deeper HIV proviral DNA testing (on PBMC) to guide either continuous ART, detect possible functional cure, or events of HIV misdiagnosis in an era of declining prevalence. (*Int J Biomed Sci* 2023; 19 (2): 31-36)

Keywords: Anti-HIV antibodies; proviral DNA; seroreversion; successful antiretroviral treatment; testing algorithm; Undetectable plasma viral load

INTRODUCTION

Over three decades, HIV epidemic remains a global challenge, with 1 million deaths due to HIV over the world, of which 310,000 in Central Africa and 29,000 in Cameroon (1). Among strategies developed to fight and curb this infection, prevention, diagnosis and antiretroviral therapy (ART) constitute the major control, surveillance and monitoring approaches. Of note, ART suppresses the viral replication (2, 3) in order to restore the specific immune response of the host (4, 5). ARV treatment is also known as a preventive tool (treatment and prevention: "TASP") (6, 7). ART has been of tremendous contribution in controlling the spread of HIV (reduction of horizontal transmission in adults, reduction of mother to child transmission), which in turn reduces risks of opportunistic infections and improves the quality of life. In this frame, the world health organisation (WHO) recommends since 2015 the strategy of test and treat, which considers ART initiation regardless of the value of TCD4+ count and viral load (8). In 2019, the joint United Nations Program on AIDS (UNAIDS) reported that 20.9 million People Living With HIV (PLWHIV) were receiving ART, this suggesting progress toward the elimination of AIDS by 2030 (9). Alongside the role played by ART in controlling disease progression, it has been observed in patients receiving early treatment the possibility of decline of humoral response, which leads to significant reduction in HIV specific antibodies, becoming undetectable below the cut-off value of *in vitro* diagnostic assays available on the market (10, 11). Of note, a loss of HIV specific antibody in an infected individual is known as seroreversion, which is basically the opposite of seroconversion. Indeed, HIV seroreversion is still unknown to several health professionals and as such case management remains very challenging to clinicians when confronted with a similar case in their routine practice. Although seroreversion is rare, ranging from 1% among adults to 7% among vertically infected children; transient or permanent, few studies have reported it worldwide (12, 14). However, knowledge on seroreversion remains very limited in settings like Cameroon, and this lack of evidence may lead to confusion during HIV management and mistaken as HIV eradication or misdiagnosis (13, 15, 16). This is particularly true for resource-limited settings (RLS) like Cameroon where seroreversion might remain uncommon. Thus, the present study aimed at evaluating rate of HIV seroreversion among ART-experienced patients with undetectable PVL in Cameroon.

MATERIAL AND METHODS

Study design

A study was carried out among HIV-infected patients reported with an undetectable PVL throughout the year 2019 at the Chantal Biya International Reference Centre for research on HIV/AIDS prevention and management (CBIRC) in Yaounde, Cameroon. Patients being monitored for their ART response at CBIRC were selected based on their virological status (PVL less than 40 copies/ml) and the availability of their archived plasma samples in the CBIRC biobank. Following written informed consent, a tested questionnaire was administered to participants, there after they were sampled for additional investigations. The study was approved by the national ethic committee n°2018/01/979/CE/CNERSH/SP. This study was conducted in accordance with the principles of the Declaration of Helsinki and the guidelines on Good Clinical Practice.

Laboratory analysis

Selected samples were re-screened for the specific anti-HIV antibodies using a serial algorithm of HIV screening as per the national guidelines. Briefly, two rapid diagnostic tests (RDTs) were used. Alere Determine HIV ½ Ag/Ab (Alere Determine, Orgenics Ltd) was used as the first test

and Oraquick Advance Rapid HIV1/2 as the second test (Orasure Technologie Inc). Those non-reactive on the first test were retested with the second test, and if non-reactive, the case was declared negative for the presence of anti-HIV antibodies.

Following application of the national algorithm for HIV screening, those non-reactive on both tests were called for further investigation. After written informed consent, 5 ml of blood were collected, and one dried blood spot (DBS) card was prepared as per the manufacturer's instructions. The new plasma of these selected participants were retested using an enzyme-linked immunosorbent assay (ELISA): RecombiLISA HIV 1+2 Ab test (CTK Biotech, Inc). These samples were tested in duplicate alongside positive and negative controls. ELISA results were expressed as optical density (OD) mean+/- STD and interpreted as either negative (if OD was less than 1) or positive (if OD was superior or equal to 1).

For molecular analysis, proviral DNA was extracted from the DBS card of patients with negative results on both RDTs and ELISA for the detection of HIV proviral DNA in their CD4 T lymphocyte cells using *gag* specific primers. A set of primers were designed based on the gag gene sequence as follow:

- CNN1: Forward 5'-AGT**G**GG**G**GGA CATCAAGCAG CCATGC-3'
- CNN2: Reverse 5' –TCCACATTTCCAACAGCCCTTTTCC-3'.

The samples were processed by Polymerase Chain Reaction (PCR) alongside positive and negative controls, and revelation was performed on an ethidium bromide agarose gel electrophoresis, with an expected band size of 682 bp. Table 1 shows the composition of the master mix and amplification conditions were as follows: one cycle of 95°C for 5 min 35 cycles of 95°C for 20 sec, 60°C for 30 sec, 72°C for 30 sec and a last cycle of 72° c for 2 min.

Table 1. Composition of the master mix for amplification

Components	25 µl	Final Concentration
10X *Taq* pol Buffer	2.5 µl	1X
10 mM dNTP	0.5 µl	200 µM
10 µM CNN1 Primer	0.75 µl	0.3 µM (0.05-1 µM)
10 µM CNN2 Primer	0.75 µl	0.3 µM (0.05-1 µM)
DNA sample template	2.5 µl	5 ng-10 ng
Nuclease-free Water	up to 25 µl	
Taq pol	0.5 µl	1 unit

RESULTS

Characteristics of the study population

A total of 546 eligible patients were enrolled in this study, consisting of 154 (27.63%) male and 392 (72.37%) female, with mean age of 41.12 years (min 3, max 78). All were receiving ART with a documented undetectable PVL (less than 40 copies/mL) and a median duration on treatment of 5 years (Table 2). The mean CD4 count was above 500 cells/mm^3.

Most of the participants (81%) were on the first line ARV regimen (2NNRTI and 1 NRTI) and on cotrimoxazole as per Cameroon national guidelines on HIV treatment and management.

Detection of anti-HIV antibodies

Following the national HIV algorithm applied on the total of 546 participants, 541 (99.1%) were reactive on the two RDTs, thus classified as seropositive for the presence of anti-HIV antibodies. Out of the 5 remaining samples, one (0.18%) sample was reactive on the first test and non-reactive on the second test, thus classified as a discordant or indeterminate result. All the 5 samples (1 indeterminate and 4 non reactive on the two RDTs) were then retested by ELISA. The indeterminate sample was positive on ELISA with an OD of 1.14 (See Table 3). The other four (0.72%) samples were confirmed to be HIV seronegative by ELISA.

Table 2. Characteristics of the study population

Description	Frequency	Percentage
Sex		
Female	392	72.37
Male	154	27.63
Age		
Min	3	Mean: 41.12
Max	78	
ART duration		
Min	1	Median duration 5
Max	16	
CD4 count		
Min	15	
Max	1852	Mean: 556

Detection of HIV proviral DNA

All the 5 samples described above were subjected to the PCR for the amplification of the *HIV gag* gene as pro viral DNA. The only patient with indeterminate/discordant results on RDTs and a seropositive result on ELISA was reported positive for proviral DNA. The rest of the four samples were tested negative for the presence of HIV gag gene. Table 3 shows testing results of the five selected patients.

DISCUSSION

The diagnosis of HIV infection through standard tests relies on the detection of anti-HIV-1/2–specific antibodies. As the virus integrate and stay permanently within the host DNA, and maintain reservoirs, HIV infection leads to permanent production of virus-directed antibodies. One exception to this is an immune dysfunction called agammaglobulinemia, which results in non-production of immunoglobulin (17, 19). Early treatment of acute HIV infection with ART may have immunologic effects on the host's cellular and humoral response. This effect, known as seroreversion (18), is a fairly rare phenomenon, suggesting that the majority of suspected cases of seroreversion may actually be due to misdiagnosis or misclassification (20). Our study has confirmed this hypothesis, as 99.1% (541) of the selected HIV-positive participants tested seropositive despite having an undetectable viral load. This result confirms that these patients are and were indeed HIV positive, and their use of ART had no significant effect on their humoral immunity (20).

As expected, only 0.7% (four) of the 546 patients presented with seronegative tests for both RDTs and ELISA, with CD4 cell counts of all four patients found to be within the normal range of 500-1200 cells/mm3. Furthermore, they did not have proviral DNA in the genome of their CD4 cells. These clinical data suggest that the immune systems of these four patients were not affected (immunocompetent), and they were falsely declared HIV positive. In order to reduce or avoid case of false positives, it is important to implement the quality management in HIV testing.

Since the sensitivity of screening tests varies from test to test, the result observed in the patient who tested negative for RDTs and positive for ELISA would be attributed to the greater sensitivity of ELISA tests compared to RDTs (21). This underscores the presence of HIV infection, as DNA-PCR was also reported to be positive. Furthermore, as the prevalence of HIV in Cameroon is declining below 5% (2.7%, HDS, 2018), it becomes crucial to consider a three-test algorithm to mitigate cases of false positives.

No case of HIV-1 seroreversion was reported by Cornelissen et al (20). Conversely, Kassutto et al (18) and Hare et al (13) identified three cases (2%) and six cases (7%) of seroreversion in their study populations (150 and 87 patients on ART, respectively). The precise meaning of the seroreversion phenomenon is still unclear, but according to some authors, it is due to a loss of antigenic stimuli resulting from the control of viral multiplication following a successful early administration of ART (18). Incomplete antibody evolution is a rare phenomenon in adults infected with HIV-1 and is linked to very early therapy (18, 22). Complete seroreversion is exceptional, and ART in this case is coupled with immunosuppressive therapy (14, 23). In children infected vertically with HIV, treated early in the first months of life, seroreversion is not rare (24, 25).

The results of our study could suggest that taking ART would not systematically induce an "alteration" of the pa-

Table 3. HIV testing results of the five selected patients

Code	Sex	Age	Determine HIV1/2	Oraquick	CD4 counts (cells/mm3)	ELISA	PCR
NESERO 1	F	30	NR	NR	574	Neg	Neg
NESERO 2	M	29	R	NR	820	Pos	Pos
NESERO 3	F	47	NR	NR	802	Neg	Neg
NESERO 4	F	25	NR	NR	564	Neg	Neg
NESERO 5	M	3	NR	NR	1035	Neg	Neg
POSITIVE C	-	-	R	R	-	Pos	Pos
NEGATIVE C	-	-	NR	NR	-	Neg	Neg

NR: Non Reactive; R: Reactive; Neg: Negative; Pos: Positive.

tient's humoral immunity and therefore seroreversion remains a rare phenomenon, probably due to late initiation of treatment in most cases. Nevertheless treatment initiation interval in respect to disease onset was not known and this data is very difficult to collect in a context of Cameroon where patients most frequently report to hospital late.

The main limitation of our study is the fact that seroreversion was investigated using proviral DNA from DBS samples, which limit the sensitivity in detecting possible HIV carriers with fewer cellular reservoirs. This therefore calls for the use of PBMC for a conclusive interpretation of such cases. Nonetheless, our evidence supports low rate of seroreversion in spite of successful ART in Cameroonian patients.

CONCLUSION

After a median of 5 years on ART, the majority of patients with an undetectable PVL remain reactive to HIV antibody testing. It appears that out of a population of 546 patients recruited in our study, no case of seroreversion was identified, however cases of false positives were potentially identified, unfortunately have been taking ART treatment over 5 years. In this prospect, using proviral DNA testing ideally on PBMC would be of great relevance to guide on continuation or discontinuation of ART in this situation.

CONFLICT OF INTEREST

The authors declare that no conflicting interests exist.

AUTHOR'S CONTRIBUTION

All authors contributed to this work.

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